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VARIATIONS IN THE DOWN COLOR OF WHITE LEGHORN CHICKS AND THEIR ECONOMIC INSIGNIFICANCE[1]

By W. F. LAMOREUX, *assistant professor of poultry husbandry*, and F. B. HUTT, *professor of animal genetics, Department of Poultry Husbandry, New York (Cornell) Agricultural Experiment Station*

INTRODUCTION

Although adult White Leghorns are all pure white (except for an occasional female with salmon breast, or a male with "brassiness" or with red on the shoulders), the down color of the chicks varies from an almost pure white in some individuals to a rich, golden yellow in others. Some poultrymen consider that the paler chicks are less vigorous and less desirable than the richly colored ones, but no actual test to determine the validity of such beliefs has hitherto been reported. Therefore, when striking differences in down color were observed in two strains of White Leghorns being selected at this laboratory for high and low requirements of riboflavin, a study was undertaken to find (1) the genetic basis for these variations, and (2) the relation, if any, of down color in the chick to such economically important characters as body size, viability, age at sexual maturity, and capacity for egg production.

MATERIAL AND METHODS

BASES OF SELECTION

In the first year of selection in Single-Comb White Leghorns for high and low requirements of riboflavin, it became evident that most of the chicks from dams considered to have a high requirement of that vitamin were darker in color than those in the "low" line. Because riboflavin is yellowish, it seemed possible that the intensity of yellow color in the down might be related to the chick's quantitative requirement of that vitamin. For that reason, all chicks in both lines were classified according to color. Later evidence showed that riboflavin had little or nothing to do with the color of the down feathers. Moreover, possibly because of difficulties inherent in the procedures used, the selection practised proved to be ineffective, and the two strains did not really differ in requirement of riboflavin. For that reason, they are hereafter designated in this paper merely as the "dark" and "light" strains.

Selection of breeders in 1936, 1937, and 1938 was based upon previous tests of their ability to reproduce when on diets deficient in riboflavin. Color of down was not considered. However, in 1939, chicks

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of the dark strain were obtained from pullets belonging to the two darkest (dams') families hatched in the previous year. Similarly, chicks of the light strain were hatched from pullets in the two lightest (dams') families of the year before.

Although eggs were incubated in two different forced-draught incubators, during any one period all eggs were placed together in the same machine, so that any changes in humidity or other environmental conditions could not affect the chicks of one strain more than those of the other.

CLASSIFICATION

For purposes of this study, the chicks were classified with respect to down color, as follows:

Dark.—Golden brown color on the back of the neck, across the shoulders, and along both sides of the breast.
Medium.—Intermediate between dark and light.
Light.—Very light cream color, sometimes with a greenish cast. White is rare.

Typical representatives of these three grades, reproduced by color photography, are shown in plate 1. In the two strains studied, there seemed to be a clear, natural distinction between dark chicks and those classified as medium or light. The medium and light chicks were therefore combined in one group so that the populations might be compared with greater ease. When this is done, any population can be briefly described by stating merely the proportion of dark chicks that it contains. On the other hand, in unselected control populations, and in the F_1 progeny from certain crosses, there was a uniform gradation in the intensity of color from dark to light. The chicks of medium color are considered separately in some of the analyses reported in this paper in order to compare either the extremes in pigmentation, or their effects. Chicks of the dark, medium, and light shades are quite indistinguishable later on when fully feathered.

A classification of colors using only three grades followed by a combination, for some purposes, of the two lighter shades may seem inadequate. It must be remembered, however, that the range of color in White Leghorns is not great. A comparison of the color standards used in the present work with those used by Warren (*11*)[2] in classifying Rhode Island Reds shows that the range of intensity of color was less in the writers' dark, medium, and light chicks than in the lightest three of his five grades.

GENETIC ANALYSIS

DOWN COLORS IN THE ORIGINAL STRAINS

The proportions of chicks in the dark and light strains that were graded as dark are shown in table 1.

It should be noted that in 1937, even though color of down had not been considered as a basis for selection of breeding stock, there was such a difference between the strains that 94.4 percent of the chicks in one strain graded as dark, while only 2.8 percent of those in the other were so classified. So far as is known, the selection practiced could have had no effect upon the segregation of genes for color. It is assumed, therefore, that the original differences between the two strains were the result of chance segregation of the genes responsible.

[2] Italic numbers in parentheses refer to Literature Cited, p. 205.

Typical representatives of dark, medium, and light colored White Leghorn chicks. In the two strains studied there seemed to be a clear, natural distinction between dark chicks and those classified as medium or light. For this reason the two lighter shades were sometimes combined to facilitate comparison.

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The rapid differentiation of the dark and light strains, without selection for color, suggests that if more than one gene is responsible for the differences in down color, they must be few in number.

TABLE 1.—*Proportions of chicks having dark down in the dark and light strains and in controls, 1936–40*

Year	Dark strain		Light strain		Unselected controls	
	Chicks classified	Chicks with dark down	Chicks classified	Chicks with dark down	Chicks classified	Chicks with dark down
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1936	42	61.9	257	19.5		
1937	124	94.4	428	2.8	827	27.7
1938	91	85.7	296	12.5		
1938S [1]	52	92.0	45	4.0		
1939	14	100.0	44	4.5		
1940	68	83.8	217	3.7		

[1] A selected population including only chicks of the 2 darkest and 2 lightest families.

After 1938, selection of breeders from the two darkest and two lightest families in the dark and light strains, respectively, effectively maintained the proportion of dark chicks at a high level in one strain and at a low level in the other. The data in table 1 show that neither strain "bred true" for its predominant shade of down, but even the exceptions of 14.3 and 12.5 percent (in 1938) are not great if one considers the difficulty of making the somewhat arbitrary classifications necessary in dealing with shades of chick down. Most of the variation occurred in a few families. Others, particularly those having darkest color, bred true to type.

In table 1, the populations labeled "1938S" include all chicks in the two darkest and two lightest families (of dams) in the dark and light strain respectively. Exceptions to the rule of color in these families were fewer than in the unselected 1938 populations from which they were taken. These few selected birds provided the parents of the 1939 generation, and these in turn were used to produce the chicks of 1940.

To determine the frequency of dark and light downs in an unselected population, 827 chicks representing four different strains of Leghorns were classified in 1937. Of these, 27.7 percent were dark, 59.6 percent medium, and 12.7 percent light. This distribution is in marked contrast to the uniformity of the dark and light strains and serves to emphasize the degree of differentiation between them.

RECIPROCAL CROSSES OF DARK AND LIGHT STRAINS

Because of the evidence, already discussed, that only a few genes were responsible for the differentiation of the dark and light strains, and the additional fact that both were breeding fairly true to type, it seemed probable that crosses between the two strains would shed some light on the genetic bases for the difference. Reciprocal crosses were made so that the effects of any sex-linked genes involved would be evident in the F_1 generation. Several different matings spread over 2 years were used for these crosses. An F_2 generation was hatched and also backcrosses of the F_1 to both the dark and light lines. The results in these various types of matings are shown in figure 1.

For simplicity, the nature of the dark and light strains, F_1, F_2, and backcross populations is shown in figure 1 merely by giving the proportion of each population that was classified as dark. The same dark females that produced the dark-strain chicks shown in figure 1 were also mated with males of the light strain (but at a different time) to produce part of the F_1 chicks considered in that figure. Similarly, the light-strain chicks and the F_1 generation from light females × dark males are all progeny of the same dams. The difference between the first and third columns in figure 1 shows, therefore, that the light-down males yielded only about two-thirds as many dark chicks as did the dark-down males when both kinds were mated to the same dark-

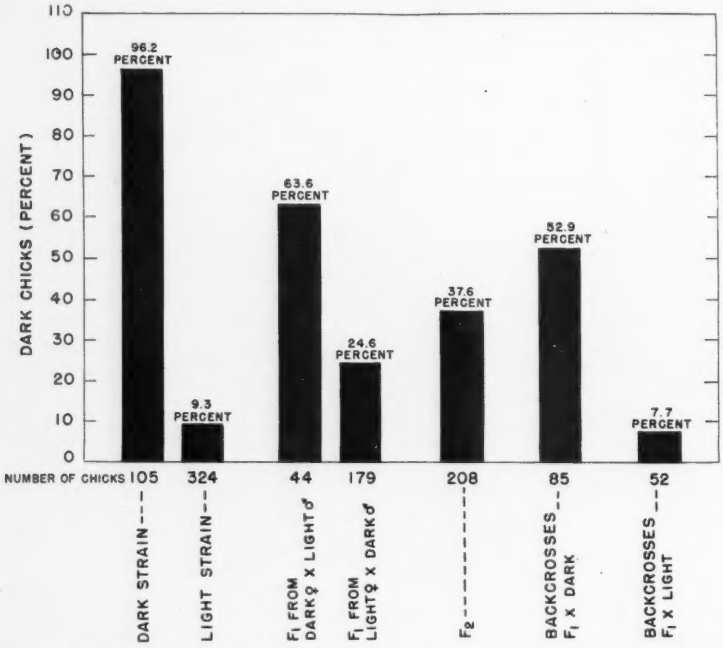

FIGURE 1.—Proportion of darkly-colored chicks in the dark and light strains, in the F_1 generations, in the F_2 generations, and in backcrosses.

down females. In the reciprocal cross, use of the dark males raised the proportion of dark chicks from 9.3 percent within the light strain to 24.6 percent in the F_1 generation.

To determine whether or not the progeny of dark males are significantly different from those of light-strain males when both are mated to the same hens, the results of such matings with 15 hens representing both strains were tabulated. The proportion of dark-colored chicks among the progeny of each of the 30 different matings was calculated in percent, and then converted to equivalent angles for statistical treatment as suggested by Snedecor (*10, p. 381*). An analysis of variance then showed that there were significantly more dark-colored chicks from the dark- than from the light-strain males, with odds of more than 100 : 1 that the difference was not due to chance.

In the two F_1 populations, the proportions of chicks with dark down were intermediate between those in the parent strains. Because of the discrepancy between the proportions of dark chicks in these two populations (63.6 and 24.6 percent), it is doubtful whether the dark and light downs can be simple allels differentiated only by a single autosomal gene. In that case, one would expect about the same proportion of dark chicks in each population.

The discrepancy between the two progenies from reciprocal crosses suggests that sex-linked genes were exerting some influence on the down color in the F_1 generation. However, if this were so, one would expect a sharp difference between the two F_1 populations of females (since in the fowl the female is the heterogametic sex) with respect to color of down.

This is illustrated by assuming a condition of incomplete dominance, but assigning to the character light down, the gene symbol L, and to dark down the symbol l. From reciprocal crosses, only one color of female could then be expected from each of the crosses as follows:

Dark female ($l-$) × light male (LL) ⟶ only light females ($L-$).
Light female ($L-$) × dark male (ll) ⟶ only dark females ($l-$).

In the first reciprocal cross (table 2, No. 1), there were obtained 21 dark females and 27 light ones. In the second cross, a great excess of light-colored females was found (table 2, No. 2) where only dark ones would be expected if the character were controlled by a sex-linked gene. These results show, therefore, that sex-linked genes have little control, if any, over the color of down, and that the differences in pigmentation of chicks obtained from reciprocal crosses must be explained in some other way.

TABLE 2.—*The colors of male and female chicks from reciprocal crosses between the light and dark strains*

Reciprocal cross No.	Mating	Males		Females	
		Dark	Medium and light	Dark	Medium and light
		Number	*Number*	*Number*	*Number*
1	Dark females × medium or light males	36	22	21	27
2	Medium or light females × dark males	17	57	16	61

In this same mating (table 2, No. 2), there was a corresponding excess of light chicks among the males, so that whatever influence the dams exerted upon the color of the progeny was approximately equal in both sons and daughters. The fact that less than 22 percent of the progeny of light dams bore dark down, while more than 53 percent of the progeny of dark dams did so (table 2), strongly suggests that some maternal influence is exerted upon the color of down in both sexes. The mechanism responsible for such an effect is unknown.

In the F_2 population as a whole, 38 percent of the 208 chicks had dark down. However, among those from a medium-down F_1 male, son of a dark dam, the proportion of dark chicks was only 23 percent, while the the F_2 progeny of a dark F_1 male from a light-strain female 60 percent were dark.

The numbers of chicks in table 2 differ from those of similar matings shown in figure 1 because different numbers of breeders were used.

Some hens were used in reciprocal matings (table 2) that were not also used for matings within their strains, as was required for figure 1.

RELATION OF DOWN COLOR TO SEX

In chicks classified as having medium down color, the ratio of males to females was almost identical with the normal expectation of 1:1 (table 3). However, among the dark chicks there was a marked excess of males, and among the light ones a corresponding excess of females (table 3).

TABLE 3.—*Distribution of down colors in relation to sex, showing an excess of males in the dark chicks and an excess of females in the light ones, 1937–39*

Population	Year	Dark		Medium		Light	
		Males	Females	Males	Females	Males	Females
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
Unselected controls	1937	165	64	257	236	40	65
Light strain	1937	6	6	135	133	63	85
	1938	29	8	106	111	13	29
F_2 generation	1939	47	21	43	52	5	13
Total		247	99	541	532	121	192
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Proportion in each color class		71	29	50	50	39	61

Down colors and sex ratios in the dark strain are omitted from table 3. Populations of that strain contained approximately equal numbers of males and females, but, since 84 percent or more of the chicks in that strain were of the darkest shade, with only 16 percent or less distributed through the medium and light shades, it could yield little information about the relation of down color to sex. The unselected control population, the light strain, and the F_2 generation showed more variability in down color. In all of these, the predominance of males among the dark chicks and of females among the light ones was consistent (table 3).

It may be noted that the populations included in table 3 contain 909 males, but only 823 females. This deficiency of females below expectation, and the discrepancies between the numbers in table 3 and those for the unsexed chicks of figure 1, probably resulted from the fact that the sex of all males was recorded when they were segregated at about 8 weeks of age, whereas the sex of females was not recorded until they were housed at about 5 months of age. By that time, their numbers had been diminished somewhat by predators. However, there is little likelihood that the females lost differed in color of down from those that survived (see table 5). For that reason, the deficiency of females cannot have affected the proportions of dark, medium, and light females given in table 3, although it has undoubtedly reduced the number in each of these classes.

Since families of dark chicks in the selected strains were differentiated from medium chicks more sharply than were the light-colored ones, there is some basis for combining the medium and light chicks in one class. When this is done, the totals for the populations given in table 3 are as follows: In males, 247 dark : 662 medium and light; in females, 99 dark : 724 medium and light.

Application of the chi square test to this distribution yields a value for χ^2 of 62 and for P of <0.001. This shows that the proportion of dark chicks among males is significantly higher than in females.

RELATION OF DOWN COLOR TO CHARACTERS OF ECONOMIC IMPORTANCE

To verify or disprove the belief of some poultrymen that the paler Leghorn chicks are less desirable than the dark ones, females of the dark and light strains, previously classified according to down color,

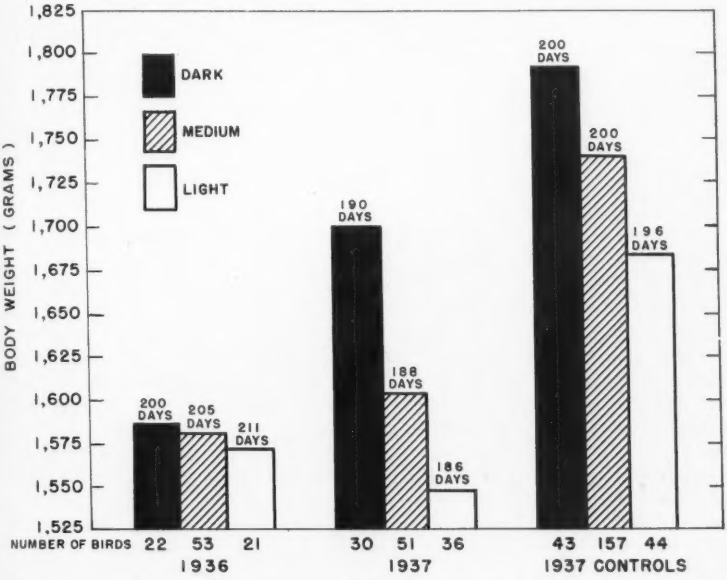

FIGURE 2.--Body weights and ages at first egg for dark, medium, and lightly colored chicks, 1936 and 1937. The numbers on the tops of the columns are the mean ages at first egg in each group.

were kept in 1936 and 1937 for measurements of their viability, productivity, body size, and age at sexual maturity. The results, presented in tables 4 to 6 and in figure 2, are not given separately for each strain, but rather for dark, medium, and light chicks as classes, regardless of the strain from which they originated. In addition, 359 chicks of the unselected control population of 1937 were utilized for similar comparisons.

BODY WEIGHT AND AGE AT SEXUAL MATURITY

It was not possible to take weights regularly of all the available birds, but, to determine whether or not the paler chicks were smaller at hatching or developed into "runts" at an early age, two lots of chicks were weighed at hatching and again at 11 days of age. The

mean weights in these two lots (table 4) show that light chicks were no smaller than the others at 11 days of age, were larger than the medium-colored ones at hatching, and smaller than the dark ones at hatching by differences that could hardly be significant with the numbers involved. Weight of the chick at hatching is highly correlated with the weight of egg. Since light and dark chicks do not differ in size, it seems improbable that the down color could be related in any way to size of egg.

TABLE 4.—*Relation of down color of chicks to weight at hatching and early growth*

Lot No.	Age	Mean weight [1] of chicks with indicated down color			Lot No.	Age	Mean weight [1] of chicks with indicated down color		
		Dark	Medium	Light			Dark	Medium	Light
	Days	*Grams*	*Grams*	*Grams*		*Days*	*Grams*	*Grams*	*Grams*
1	1	36.5	33.2	33.8	2	1	36.7	35.4	36.2
	11	65.0	63.1	65.0		11	68.5	57.3	69.1

[1] 15 to 75 chicks per group.

Weights at sexual maturity in three separate lots of birds were consistently highest for the dark chicks and lowest for the light ones (fig. 2). Chicks of medium down color had body weights intermediate between those of the other two classes. The consistent association of larger size with darker down in all three classes looks significant. However, further study of these data by the analysis of covariance (*10*) showed that in the birds of 1936 and in the 1937 controls there existed no significant differences in body weight when the groups were adjusted to a common basis with respect to age at first egg. A value for P of <0.05 was obtained with the data from 1937. This value is often used to show that a significant difference does exist, but in view of the fact that significant differences were not found in the other two populations, one is forced to conclude that no important relationship between color of down and body weights exists in these data.

VIABILITY

Mortality from hatching to 160 days of age, when pullets were put in winter quarters, did not differ significantly or consistently in dark, medium, and light chicks of three separate lots (table 5).

TABLE 5.—*Independence of down color and viability in White Leghorn pullets to 160 days of age, 1936 and 1937*

Year	Total chicks in all 3 down-color classes	Mortality to 160 days of age			Total pullets in all 3 down-color classes	Mortality from 160 days to Jan. 23 [1]		
		Dark down	Medium down	Light down		Dark down	Medium down	Light down
	Number	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*
1936	175	14	18	18	142	10	12	26
1937	177	23	21	21	139	9	15	11
1937, controls	359	11	12	9	250	[2] 42	[2] 46	[2] 38

[1] This period had an average length of 122 days in 1936 and 108 days in 1937.
[2] Mortality from 160 to 500 days of age.

For two of these lots, comprising pullets hatched in 1936 and 1937 from the dark and light strains, subsequent mortality was recorded from 160 days of age to January 23 of the following year. In 250 unselected control pullets of 1937, mortality was recorded from 160 days to 500 days of age. The figures, given in table 5, show that mortality differed slightly in the dark, medium, and light chicks, probably by chance and because of small numbers in some classes, but was not consistently higher in any one color class. In the largest lot, the 1937 controls, mortality from housing time to 500 days of age was 4 percent less in 65 pullets originally having light down than in 81 birds that had the darkest down when hatched. Moreover, considering only the pullets in this lot that died after 160 days of age, the mean ages at death for birds of dark, medium, and light downs were respectively 368, 387, and 390 days. These differences in favor of the light-down pullets are not significant, but they do show that viability in such birds was equally as good as in those that originally had dark down.

CAPACITY FOR EGG PRODUCTION

TABLE 6.—*Independence of down color and egg production in White Leghorn pullets, 1936 and 1937*

Year	Period of test, from first egg to—	Pullet egg production for indicated chick down color					
		Dark		Medium		Light	
		Pullets	Mean eggs	Pullets	Mean eggs	Pullets	Mean eggs
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
1936	Feb. 1, 1937	23	38	54	33	23	30
1937	Jan. 23, 1938	21	42	41	58	26	55
1937, controls	500 days of age	47	162	56	160	40	166

The relation of down color to productivity was studied in the same three populations as were used to study viability. The results, given in table 6, show no relation whatever between ability to lay eggs and color of down. By chance, the most productive class had dark down in one lot, medium-color down in another, and light down in the third. In the longest and most significant test, that of the 1937 controls, pullets that originally had light down laid four eggs per bird more than those that had dark down as chicks. The difference is quite insignificant but this test does show that the light chicks are not inferior to the dark ones.

IS THE YELLOW COLOR RELATED TO RIBOFLAVIN?

Because the light and dark strains of White Leghorns were differentiated while selecting for high and low requirements of riboflavin, which has a yellow color, it was desirable to determine to what extent differences in content of riboflavin were associated with differences in color of the down. The yellow pigment in the down of White Leghorn chicks is not soluble in petroleum ether, or in chloroform. It is partially dissolved, but not completely so, by digestion for 1 to 2 hours in a mixture of 25 percent normal sulfuric acid and 75 percent acetone. The extracts used in this study were obtained by this method.

Assays of the riboflavin content of these extracts were made in two ways. Photometric analyses of extracts from down collected in 1937 showed that the dark down contained the most riboflavin and the light down the least. Other samples taken in 1940 were kindly assayed by Dr. A. Z. Hodson according to the improved method of Hodson and Norris (*4*). The average content of riboflavin in two series of dark, medium, and light downs was respectively 3.9, 2.5, and 1.8 micrograms per gram of down. Since these are much smaller amounts of riboflavin than are found in red muscle or in glandular tissues, the concentration of that substance in the down is comparatively low.

It seems unlikely, therefore, that the differences in down color are related to riboflavin. The method used for its extraction is considered to remove most of the riboflavin, but even after its extraction the differences between the dark, medium, and light downs were practically as conspicuous as before extraction, despite marked coloration of the extract from the dark down. Furthermore, when the extracts were treated with a reducing solution of sodium hydrosulfite and sodium bicarbonate, the brownish pigment in them was not reduced to a colorless form as riboflavin usually is when so treated. For all these reasons, it seems improbable that differences in color of the down were dependent upon the presence of different amounts of riboflavin.

HEAD SPOTS

RELATION OF HEAD SPOTS TO COLOR OF DOWN

In contrast to chicks of most other breeds, White Leghorns are conspicuously lacking in patterns or stripes in the down. The nearest semblance to a pattern is a dark, brownish spot occurring on the back of the head. Some of these spots are quite large and conspicuous, others barely visible. Difference in size of the spot depends, not upon intensity of the color, but upon the number of long strands of pigmented down. Some of these include only two or three dark-colored strands and are barely evident. These head spots in White Leghorns are quite different from those of Rhode Island Reds, where a black or brown spot contrasts sharply with the lighter-colored, adjoining down.

TABLE 7.—*Distribution of head spots among White Leghorn chicks having down of dark, medium, or light color*

Population	Proportion of chicks with head spots in groups having indicated down color							
	Dark		Medium		Light		3 down-color classes combined	
	Total	With head spots	Total	With head spots	Total	With head spots	Total	With head spots
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Dark strain, 3 years	209	99	20	100			229	99
Light strain, 3 years	51	76	521	26	196	51	768	36
Controls, 1937	229	96	493	77	105	27	827	76
F_2 generation from cross light × dark	68	91	95	74	18	39	181	77

The distribution of head spots in relation to color of down is shown in table 7. Head spots occurred in nearly every chick of the dark strain, but only in 36 percent of those in the light strain. The difference results in part from the fact that head spots are difficult to recognize on lightly colored chicks. However, because even in the dark chicks of the light strain the proportion showing head spots was lower (76 percent) than in dark chicks of the other populations, it seems probable that the genes causing light down may also tend to eliminate head spots. In the F_2 generation, the distribution of head spots was practically the same as in the unselected control population of 1937, both having head spots in about the same proportion of each color class and in about three-quarters of all chicks.

RELATION OF HEAD SPOTS TO SEX

In the control population, the light strain, and the F_2 generation, males comprised 71 percent of the dark chicks and females 61 percent of the light ones (table 3). Since head spots are found in over 90 percent of unselected dark chicks, but in less than 40 percent of the light ones (table 7), it follows that if all chicks in any population are considered, there must automatically be a preponderance of males among those with head spots, and of females among those without them. However, there is evidence that, apart from this spurious relationship, and regardless of down color, head spots are more frequent in males than in females. In the light strain and the unselected controls (table 7), there were 1,014 chicks of known sex classified as having down of medium color. The sex ratio in these—514 males : 500 females—was normal. In this population, the distribution of sexes and head spots was as follows:

	Males	*Females*
Chicks with head spots	288	225
Chicks without head spots	226	275

Application of the chi square test to this distribution yields a value for χ^2 of 12.34 and for P of <0.001. Since the probability of such a distribution occurring by chance is less than 1 in 1,000, it is apparent that among chicks bearing head spots the proportion of males is significantly greater than among chicks lacking them.

This contrasts with the situation in Rhode Island Red chicks where, as Byerly and Quinn (*1*) and Hays (*3*) have shown, over 82 percent of the chicks with black head spots are females. In that breed, however, the spot is quite different in color, size, and shape from those found in White Leghorns.

DISCUSSION

Since the introduction by Punnett and Pease (*9*) of the Cambar, a breed of fowls in which the sexes of the chicks are recognizable at hatching because the females are darker than the males, the colors and markings of the down feathers of chicks have been studied by several investigators. Hagedoorn (*2*), Lamoreux (*7*), Jaap (*5*), and Punnett (*8*) have established new "autosexing" breeds in which, as in the Cambars, identification of sex at hatching is facilitated by a light ground color or pattern in the down which accentuates the normal difference between males homozygous for barring and females hemizygous for that sex-linked character. Even in pure Barred Plymouth

Rocks there are differences in color between males and females. Jerome (*6*) was able to identify sex in that breed with an accuracy of 98 percent by considering differences in the head spot, the color of down, and the color of shanks and feet.

In White Leghorns, as in all these autosexing breeds and in Barred Rocks, the males are homozygous for barring and the females are hemizygous, but these conditions apparently do not cause corresponding differences in color. In fact, since males predominate among dark chicks and females tend to have light down (table 3), what difference does occur is exactly the opposite of that in Barred Rocks and in the autosexing Cambars, Barnevelders, Ancobars, Oklabars, and Legbars, where the males are light and the females dark. Apparently any effect of the barring gene is as effectively masked by dominant white in chicks as it is in adult White Leghorns. Identification of sex by color of the down is impossible in White Leghorns, except for the generalization that the darkest chicks are more likely to be males and the lightest ones to be females.

SUMMARY

From a stock of White Leghorns there were differentiated two strains, in one of which about 83 to 94 percent of the chicks had dark down, while in the other only 3 to 12.5 percent had dark down, the remainder grading as medium or light in color. Because this sharp difference between the strains was established in 2 years without deliberate selection for color, and was subsequently easily maintained, it is considered that only a few genes caused the difference between the dark and light strains.

In F_1 generations from reciprocal crosses between these two strains, the proportion of dark chicks was intermediate between those in the parent strains. However, among the F_1 progeny from dark dams there was a higher proportion of dark chicks (63.6 percent) than in F_1 chicks from light dams (24.6 percent).

It was shown that sex-linked genes were not primarily responsible for differences in down color. The tendency for the F_1 progeny from reciprocal crosses to resemble the dam's strain more than that of the sire is attributed to some kind of maternal influence, the basis for which is as yet unknown.

In an unselected control population, the proportions of dark, medium, and light chicks were 28, 59, and 13 percent respectively. In chicks of medium color, the proportions of males and females were about equal. Of the dark chicks, 71 percent were males; and of the light ones, 61 percent were females.

Color of down was shown to be quite unrelated to size at hatching, early growth, age at sexual maturity, viability, and ability to lay eggs. In weight at first egg, the darker chicks were consistently the heaviest, and the paler chicks the lightest, but it is not clear that these associations are significant.

The amount of riboflavin was highest in dark down, and lowest in light down, but it is considered that down color is not determined by that substance.

Dark spots in the occipital region of the head occurred in 99 percent of chicks in the dark strain, 36 percent of those in the light strain, and 76 percent of unselected control chicks. In all strains they were

most frequent in dark chicks and least so in light ones. They were more frequent in males than in females.

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SIMULTANEOUS MEASUREMENT OF CARBON DIOXIDE AND ORGANIC VOLATILES IN THE INTERNAL ATMOSPHERE OF FRUITS AND VEGETABLES [1]

By FISK GERHARDT

Physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Maturity, handling, storage, and ripening induce changes in the metabolism of fruits and vegetables, which are reflected in the composition of their internal atmospheres. Quantitative analysis of such atmospheres should be of value in problems connected with gas storage, waxing, storage scald, core break-down, fungus decay, staleness, and the development of off-flavors.

The organic volatiles in such atmospheres contain certain odorous constituents, such as the amyl esters of formic, acetic, caproic, and caprylic acids (*12*),[2] together with acetaldehyde (*8*, *10*), ethyl alcohol (*6*), ethylene (*9*), and essential oils. The common inert atmospheric gases are also present. As carbon dioxide is an end product of respiration, changes in the concentration of this gas assume special importance.

In a previous publication (*7*) Gerhardt and Ezell presented a method of estimating the organic volatiles emanating from stored fruit. The present paper describes a method of measuring simultaneously the total oxidizable volatiles and the carbon dioxide present in the internal atmosphere of fruits and vegetables. The method involves the refluxing of the plant tissue in an aeration stream of carbon dioxide-free air, the absorption of the organic volatiles in concentrated sulfuric acid and of the carbon dioxide in 0.1 N barium hydroxide, and the oxidation of the volatiles with 0.1 N ceric sulfate. The procedure permits the use of inexpensive laboratory equipment and lends itself to routine analysis with sufficient accuracy for most purposes.

REVIEW OF METHODS FOR MEASURING INTERNAL ATMOSPHERE IN FRUITS

CARBON DIOXIDE

Most of the methods for measuring the carbon dioxide content of the internal atmosphere in plant products have been fundamentally similar in principle, in that segments of tissue were surrounded with mercury and, after subjection to a vacuum, the expelled gases were collected, measured, and analyzed. Magness (*11*), using this principle, devised an extraction chamber and developed a technique of analysis employing the Bonnier-Mangin gas apparatus. Later Culpepper and coworkers (*5*), and more recently Brooks (*1*), employed

[1] Received for publication April 30, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 218.

enlarged extraction chambers and obtained sufficient quantities of gas to permit the use of the Orsat type of apparatus for analysis.

Although these methods have been used extensively and perhaps are suitable when comparing fruits of one kind, they may not give a true picture of the internal atmosphere. The total carbon dioxide in the tissue undoubtedly is present in different forms; namely, (1) as gaseous carbon dioxide in the intercellular air spaces and (2) as carbon dioxide in solution in the cell sap. Since the concentration of the dissolved gas in solution is directly proportional to the concentration in the free space above the liquid (Henry's law), application of a partial vacuum would immediately upset the equilibrium of such a system. Both Magness (*11*) and Brooks (*1*) recognized this fact in interpreting the limitations to be placed on the vacuum method as a measure of the total carbon dioxide in the internal atmosphere.

Willaman and Brown (*16*) studied the relationship between dissolved carbon dioxide and respiration in apple twigs, in which the total internal carbon dioxide was measured. Their method consisted essentially in submersion of the sample in boiling 95 percent ethyl alcohol in a closed container and removal of the carbon dioxide under reduced pressure by the use of CO_2-free air. The expelled carbon dioxide was entrapped in a standard alkali solution. Claypool (*4*) used a modification of this principle wherein the total internal carbon dioxide was measured by boiling the tissue in 200 cc. of distilled water for 15 minutes in a 1-liter Erlenmeyer flask. The liberated gases were swept out of the apparatus with CO_2-free air, and the carbon dioxide was absorbed in half-normal sodium hydroxide.

Wardlaw and Leonard (*15*) used a simplified modification of the method of Willaman and Brown (*16*) for measuring the total internal carbon dioxide in the banana. Their paper included an excellent diagrammatic description of the apparatus and its manipulation. Distillation flasks, condensers, and traps were fitted with ground-glass joints. The fruit tissue was immersed in boiling alcohol and aerated with CO_2-free air, and the liberated carbon dioxide was entrapped in Pettenkofer tubes carrying barium hydroxide. The variation of replicate samples was approximately 6 percent; loss of carbon dioxide, due to sampling, was of similar magnitude. Acidulation of the alcohol for extraction was found unnecessary, as any carbon dioxide present in combined form was liberated as a result of boiling with the acids already present in the tissue. Asymptotic curves, representative of carbon dioxide liberation, were obtained during reflux aeration of the normal alcoholic blank, from the alcohol charged with carbon dioxide, and also from green and ripening fruit tissue.

ORGANIC VOLATILES

Reference should be made to a recent publication by Gerhardt and Ezell (*7*) for a review of methods of measuring certain odorous constituents emanating from fruits and vegetables. Obviously, if these substances are liberated from the surface of intact fruits, they must exist in larger amounts in the internal atmosphere and cell contents of such fruits. Steam distillation of the tissue has been used as a general procedure for the removal of these volatiles.

Acetaldehyde (*6*, *8*, *10*) in the steam distillate has usually been trapped as the sodium bisulfite addition compound and determined

by iodometric means, whereas ethyl alcohol (*6*) has been oxidized to acetic acid with a sulfuric acid-potassium dichromate mixture. Christensen and coworkers (*3*) developed a new apparatus for the extraction of internal gases from plant tissue in connection with a bromination micromethod for the accurate determination of ethylene within a range of 0.001 to 0.06 cc. in an air sample of 35 to 40 cc.

Concentration of the steam distillates of the pulp and peelings of McIntosh apples by Power and Chesnut (*12*) produced pale-yellow viscous essential oils of pleasant applelike odor. These substances were present to a greater degree (0.0022 percent) in the parings than in the pulp (0.0012 percent). Hydrolysis of the esters in the distillates produced amyl alcohol and substances of roselike fragrance suggestive of geraniol. Oxidation of these alcohols led to the formation of valeric acid, acetone, and levulinic acid. The presence of the last two oxidation products, together with the purple coloration with Schiff's reagent, served to identify this volatile of roselike odor as the aliphatic terpene, geraniol.

EXPERIMENTAL PROCEDURE

PURPOSE AND BASIC PRINCIPALS OF PROPOSED METHOD

In connection with certain previous studies on the physiology of storage scald of apples, a method was devised for measuring the total volatile emanation from stored fruit (*7*). The rate of emanation of these volatiles from fruit in cold storage was often very low. It was recognized that, if these components could be measured as a group in the internal atmosphere of the fruit itself, their assay might be of greater importance to the solution of the problem.

The present method was evolved by combining a modification of the extraction technique of Wardlaw and Leonard (*15*) for the removal of total carbon dioxide from internal atmospheres, with the procedure described by Gerhardt and Ezell (*7*) for the absorption of volatile emanations from stored fruit. Briefly, it consists of refluxing the tissue in boiling distilled water; aerating with CO_2-free air; and passing the liberated gases through dual scrubbers, the first one containing concentrated sulfuric acid for the absorption of organic volatiles and the second containing 0.1 N barium hydroxide for the removal of carbon dioxide. Carbon dioxide and volatiles in the internal atmosphere of plant tissue are thereby measured simultaneously from the same sample in one operation.

DESCRIPTION OF APPARATUS

Figures 1 and 2 show the main features of the apparatus. The digestion equipment (fig. 1) consists essentially of the following parts: A 1-liter round bottom distilling flask with interchangeable ground-glass gas inlet tube, a soda lime trap and a Drechsel gas washing bottle carrying concentrated sulfuric acid, and a 10-inch Allihn reflux condenser bearing a Kjeldahl distillation trap and glass tubing for connection with the absorption towers in figure 2.

The essential features of the absorption and aeration equipment in figure 2 consist of the Truog absorption towers and the flowmeter. The towers of heavy glass tubing contain glass beads and are stoppered into 250-cc. side-arm extraction flasks. The first tower attached

to the extraction apparatus is 10 mm. in diameter, uses 2- to 3-mm. glass beads, and contains concentrated sulfuric acid for the removal of volatiles. The second tower is 20 mm. in diameter, uses 5- to 6-mm. glass beads, and contains 0.1 N barium hydroxide for the removal of carbon dioxide. The flowmeter is calibrated for 20 liters

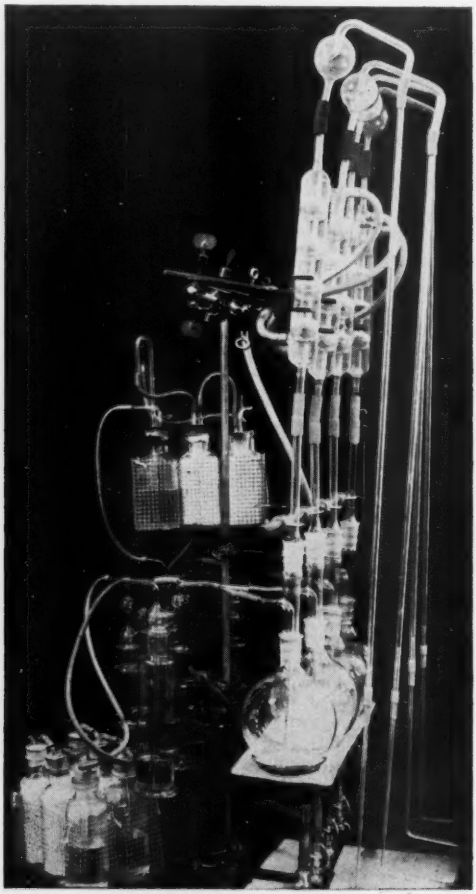

FIGURE 1.—Digestion and reflux apparatus for extraction of carbon dioxide and volatiles in the internal atmosphere of plant tissues.

of air per hour and connects the second tower with the source of aspiration through the surge chamber and mercury trap.

STANDARDIZATION OF TECHNIQUE

ABSORBENTS, OXIDATION, AND TITRATION OF ABSORBATES

In previous work (7), concentrated sulfuric acid (specific gravity, 1.84) was employed to absorb the volatile emanations from fruit.

Ethylene, however, is not appreciably absorbed in this reagent. Tropsch and Mattox (*14*) used sulfuric acid activated with nickel and silver sulfates to remove ethylene from gaseous hydrocarbons. This activated acid mixture, in the proportion of 1 part of concentrated sulfuric acid (specific gravity, 1.84) saturated with nickel sulfate at room temperature to 15 parts of concentrated sulfuric acid containing 0.6 percent of silver sulfate, was used in the present studies. Ten cubic centimeters of this acid mixture was employed

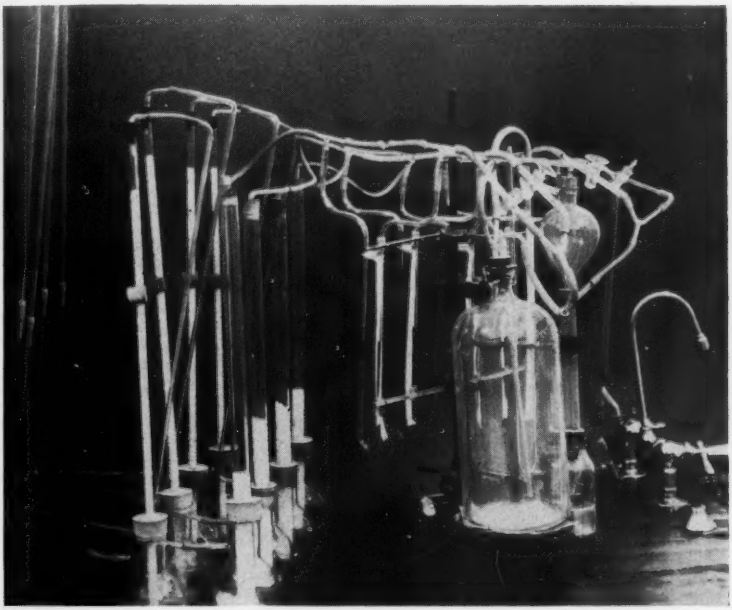

FIGURE 2.—Absorption and aeration equipment for measuring carbon dioxide and volatiles in the internal atmosphere of plant tissues; left to right, sulfuric acid tower for removal of volatiles, barium hydroxide tower for removal of carbon dioxide, flowmeter, surge chamber, and mercury trap.

for absorption of volatiles, and 25 to 50 cc. of 0.1 N barium hydroxide for the removal of carbon dioxide.

The oxidation of the volatiles with 0.1 N ceric sulfate dissolved in 0.5 M sulfuric acid was carried out as previously recommended by Gerhardt and Ezell (*7*). It entailed heating at 98° to 100° C. for 2 hours in a solution of at least 1 molar concentration in sulfuric acid. The amount of ceric sulfate reduced in the oxidation of the volatile material was determined by titration against 0.1 N ferrous ammonium sulfate, 0.005 M orthophenanthroline ferrous complex (*13*) being used as an indicator. Volatiles were expressed in terms of milligrams of ceric sulfate reduced per 100 gm. of tissue. Carbon dioxide, as milligrams per 100 gm. of tissue, was determined by titration of the reserve barium hydroxide in the absorption tower with 0.1 N hydrochloric acid, phenolphthalein being used as an indicator.

Blank or Check Runs

Some carbon dioxide is initially present in the air within the extraction apparatus, and a certain amount of necessary contamination also occurs during dismantling of the absorption tower prior to titration of its contents. Slight reduction of ceric sulfate due to aeration and other manipulative practices should also be anticipated. These factors were studied by running a series of blank determinations. One hundred cubic centimeters of CO_2-free distilled water was added to each distillation flask. The absorption towers contained 10 cc. of activated sulfuric acid and 25 cc. of 0.1 N barium hydroxide. The boiling water in the distillation flasks was aerated at a rate of 20 liters per hour for 2 hours with CO_2-free air. At the close of the extraction period, any absorbates present were oxidized and titrated as described in the previous sections. The results are presented in table 1. These average corrections of 0.72 cc. and 0.25 cc. for carbon dioxide and volatiles, respectively, were used in all ensuing studies.

Table 1.—*Blank determinations by the proposed method for the analysis of internal atmospheres*

For carbon dioxide			For organic volatiles		
$Ba(OH)_2$ (0.1 N) taken	HCl (0.1 N) back titer	Correction as indicated by $Ba(OH)_2$ (0.1 N) used (blank)	$Ce(SO_4)_2$ (0.1 N) taken	$Fe(NH_4SO_4)_2$ (0.1 N) back titer	Correction as indicated by $Ce(SO_4)_2$ (0.1 N) reduced (blank)
Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
27.37	26.65	0.72	25.10	24.80	0.30
27.37	26.65	.72	25.10	24.85	.25
27.37	26.70	.67	25.10	24.85	.25
27.37	26.60	.77	25.10	24.90	.20
Average		.72	Average		.25

Extraction Period for Internal Atmospheres

The influence of the length of the extraction period on the yield of volatiles and carbon dioxide was also determined. Two hundred grams of tissue of fresh Delicious apples was placed in each digestion flask along with 100 cc. of CO_2-free distilled water. The rest of the procedure was similar to that described for the determination of blanks. At intervals aeration was interrupted momentarily to insert fresh absorption towers into the hook-up. Data in table 2 show the amount of carbon dioxide and volatiles liberated at specific intervals over a period of 10 hours of extraction.

Table 2.—*Influence of length of extraction period on the analysis of carbon dioxide and organic volatiles in the internal atmosphere of tissue of Delicious apples*

Digestion period (hours)	Total CO_2 liberated per 100 gm. of fresh tissue	Amount of CO_2 liberated per additional period	Total $Ce(SO_4)_2$ reduced per 100 gm. of fresh tissue	Amount of $Ce(SO_4)_2$ reduced per additional period
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
1	4.40		94.2	
2	8.95	4.55	166.0	71.8
4	10.56	1.61	192.6	26.6
6	12.15	1.59	215.7	23.1
8	13.60	1.45	237.3	21.6
10	15.10	1.50	256.5	19.2

There was a large increase in the liberation of these constituents between the first and second hours of extraction; thereafter, the additional amount removed in general grew progressively less with each 2-hour period of extraction. Undoubtedly the liberation of volatiles by steam distillation occurs at an asymptotic rate, although the limited data in figure 3 do not show this relationship. The sharp reduction in the quantity of volatiles removed after the second hour of extraction

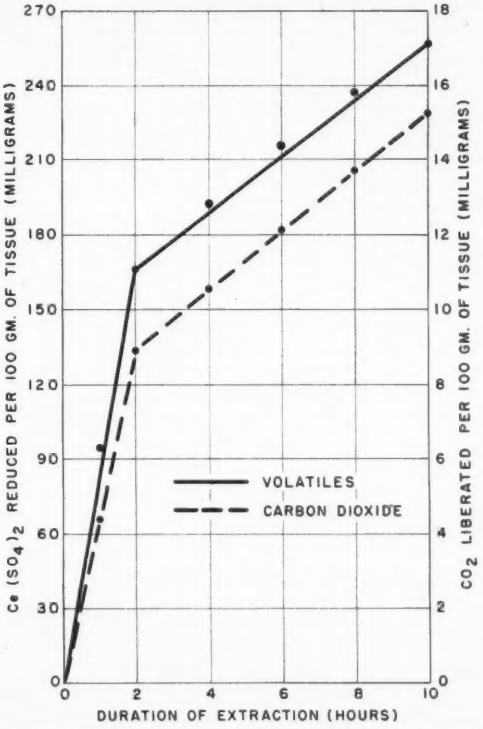

FIGURE 3.—Relationship between length of extraction and liberation of volatiles and carbon dioxide from Delicious apple tissue.

is substantiated by the experience of Wardlaw and Leonard (*15*) and also by that of Willaman and Brown (*16*) in their removal of carbon dioxide from plant tissues by aspiration in boiling alcohol. For all ensuing studies the period of extraction has been standardized to 2 hours of reflux and aeration.

INFLUENCE OF SAMPLING TECHNIQUE

One common lot of Delicious apples (sample A) and one of Winesap (sample B) previously stored at 31° F. were used in the study. Ten fruits of each variety were cored, quartered, and finely minced in a food chopper. A similar series of 10 fruits each were cored by passing a 1-inch cork borer from the calyx through to the stem of each fruit.

Samples of tissue were then removed for analysis by passing a ¾-inch cork borer through the greatest transverse diameter of the fruit. The plugs of fruit tissue were then transferred directly into the tared distillation flask of the apparatus. Two hundred grams of tissue per flask was used in each method of sampling. The remaining features of the technique were standardized as previously described.

The results, shown in table 3, indicate that considerable amounts of carbon dioxide were lost when the tissues were minced during sampling. The amount of volatile material, however, was not greatly influenced by either type of procedure. The losses in carbon dioxide were considerably greater than those reported by Wardlaw and Leonard (*15*), who permitted ground tissue to stand for different periods of time prior to immersion in the extraction apparatus. Evidently the major portion of the carbon dioxide loss occurs during grinding of the tissue and not during subsequent delay. Speed and adaptability recommend the cork-borer technique as the preferable means of sampling fleshy fruits and vegetables.

TABLE 3.—*Influence of sampling technique on the analysis of carbon dioxide and volatile material in the intercellular atmosphere of tissue of Delicious and Winesap apples*

Sampling procedure	CO_2 per 100 gm. of fresh tissue	Organic volatiles expressed as $Ce(SO_4)_2$ reduced per 100 gm. of fresh tissue
	Milligrams	*Milligrams*
Minced in food chopper:		
Delicious apples (sample A)	6.66	171.0
Winesap apples (sample B)	3.97	105.8
Cylinders of tissue removed by ¾-inch cork borer:		
Delicious apples (sample A)	10.04	168.0
Winesap apples (sample B)	7.56	108.7

EFFICIENCY OF METHOD

The accuracy of the proposed technique for the estimation of volatile constituents was tested by trial recovery of known amounts of acetaldehyde. Approximately 5 to 13 mg. of this volatile was added to the distillation flasks, and the analyses were carried out as recommended. Equivalent amounts of acetaldehyde were calculated from the milligrams of ceric sulfate reduced (based on a reduction-oxidation ratio of ceric sulfate to acetaldehyde of 51:1 as found by Gerhardt and Ezell (*7*)).

The results of this study are shown in table 4. They indicate that approximately 91 percent of the total acetaldehyde is recovered during the 2 hours of extraction as recommended in the proposed method. The rate of acetaldehyde removal from aqueous solution, like that of volatiles from Delicious apple tissue, is probably asymptotic in nature. Therefore, each increment of acetaldehyde recovered would require a progressively longer period of extraction, and beyond 91-percent recovery, further extraction would hardly be feasible for practical application.

A total recovery of 91 percent does not indicate a 9-percent variation between individual analyses of comparable samples. In such instances one should anticipate a variation of approximately 1 percent,

as shown in table 4. Analyses of such heterogeneous mixtures of volatiles as are found in the odorous constituents of plants hardly presuppose the accuracy of measurement possible with many inorganic compounds.

TABLE 4.—*Recovery of acetaldehyde by the proposed method for the analyses of internal atmospheres*

Acetaldehyde added (milligrams)	$Ce(SO_4)_2$ (0.1 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Acetaldehyde	Recovered
	Cubic centimeters	*Cubic centimeters*	*Milligrams*	*Milligrams*	*Percent*
5.28	24.9	7.4	245.8	4.81	91.1
10.56	24.9	14.7	489.3	9.59	91.0
13.20	24.9	18.6	617.9	12.11	91.7
Average					91.3

RÉSUMÉ OF DETAILS FOR PROPOSED METHOD

Sampling.—The cork-borer technique as previously described is recommended for sampling. The wall of the borer and the excised portion of peeling form a seal for the sample, which minimizes contamination with the atmosphere. The plugs of tissue are ejected directly into the tared 1-liter distillation flask containing 100 cc. of CO_2-free distilled water.

Extraction and absorption of internal gases.—The extraction and absorption apparatus is assembled as previously described in the text and in figures 1 and 2. Usually 200 gm. of plant tissue is then refluxed and aspirated for 2 hours with CO_2-free air at the rate of 20 liters per hour. At the close of the extraction period the towers are dismantled and the glass beads are washed into the extraction flasks with CO_2-free distilled water (25 cc. for the acid tower and 50 to 75 cc. for the alkali tower).

Oxidation and titration of absorbates.—Twenty-five to 50 cc. of 0.1 N ceric sulfate solution (depending on the amount of volatiles expected) is added to the extraction flask containing the absorbed volatiles. Oxidation, titration technique, and expression of results for both volatiles and carbon dioxide follow the procedure previously described in this paper.

APPLICATIONS OF THE METHOD

Brooks, Cooley, and Fisher (*2*) suggested that the effectiveness of oiled wraps for control of common storage scald in apples may be due "to their power of absorbing esters or other similar products thrown off in gaseous form by the apple." By use of the proposed method, a test was made of the adsorptive capacity of plain and oiled paper wrappers for the odorous emanations from ripening fruit. Unwrapped Delicious apples, after 135 days' storage at 31° F., were removed to a ripening room at a temperature of 65°. One lot of fruit was wrapped in oiled paper (18.7 percent oil) and a second lot in plain paper wraps. After 10 days of ripening, the fruit wraps from both lots were removed and analyzed for their content of total volatiles. It was found that the oiled wraps contained 35.37 mg. of volatiles per wrap, whereas the plain paper carried only 3.82 mg. In other words, the oiled

paper took up approximately 10 times as much of the volatile emanations from apples as did the plain paper fruit wrap.

The influence of storage temperature on the accumulation of volatiles in the internal atmosphere of Winesap and Delicious apples was also studied. Data pertaining to this experiment are summarized in table 5. Total volatiles were calculated in terms of milligrams of both ceric sulfate reduced and of acetaldehyde. Furthermore, acetaldehyde was determined separately by steam distillation (*6*) of samples comparable to those used for the estimation of total volatiles. By this dual procedure, it was possible to show the proportion of the acetaldehyde to total volatiles and also how this relationship varied with the duration and temperature of storage.

TABLE 5.—*Influence of storage temperature on the accumulation of volatiles in the internal atmosphere of Winesap and Delicious apples*

[All lots of fruit wrapped in oiled paper; no scald present at the time of sampling]

Variety	Storage temperature	Length of storage	Total volatiles per 100 gm. of fresh tissue expressed as—		Acetaldehyde per 100 gm. of fresh tissue by actual analysis	Total volatiles due to acetaldehyde
			$Ce(SO_4)_2$ reduced	Acetaldehyde		
	° F.	*Days*	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*
Delicious	(1)	0	69.7	1.3	0.16	12.3
Do	31	180	148.9	2.9	.58	20.0
Do	31	225	240.8	4.7	1.14	24.2
Do	36	[2] 180	268.0	5.2	2.37	45.6
Do	36	[2] 225	513.5	10.0	4.97	49.7
Winesap	31	154	90.7			
Do	[3] 30–40	154	132.2			
Do	36	154	260.7			

[1] At harvest.
[2] Abnormal, stale, acetaldehydic flavor.
[3] Commercial storage.

The data in table 5 show that both total volatiles and acetaldehyde accumulate in the internal atmosphere of Delicious apples during storage. Furthermore, acetaldehyde evidently accumulates in greater amounts than other odorous constituents, since it forms an increasingly larger proportion of the total volatiles as the storage period is lengthened. After storage at 36° F. for 225 days, acetaldehyde accounts for approximately 50 percent of the total volatiles, and the flavor is definitely impaired. Previous unpublished studies have also shown a positive correlation between acetaldehyde accumulation and the development of stale off-flavors in the Delicious apple. In Winesap as in Delicious apples, there is also a greater accumulation of total volatiles during storage at 36° than at 31°.

Analyses of the internal atmosphere of a miscellaneous group of fruits and vegetables are shown in table 6. Ripening processes produced a much greater change in the internal atmosphere of the Bartlett pear than in that of the Golden Delicious apple. The increase in carbon dioxide during ripening amounted to approximately 65 percent in the pear and only 30 percent in the apple, whereas the organic volatile content increased more than tenfold in the former and only about 12 percent in the latter. A greater proportion of the total volatiles was composed of acetaldehyde in the Bartlett pear than

in the Golden Delicious apple, and, furthermore, the increase of acetaldehyde during ripening was approximately 80 times as great in the pear as in the apple tissue. After ripening at 65° F. for 7 days, the pear fruits were rather severely scalded, and the large increase in acetaldehyde during ripening may have been closely associated with the presence of this storage disorder, as previously reported by Harley and Fisher (*10*).

TABLE 6.—*Analyses of the internal atmosphere of different fruits and vegetables*

Type of sample	CO_2 per 100 gm. of fresh tissue	Total organic volatiles per 100 gm. of fresh tissue expressed as—		Acetaldehyde per 100 gm. of fresh tissue by actual analysis	Total volatiles due to acetaldehyde
		$Ce(SO_4)_2$ reduced	Acetaldehyde		
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*
Bartlett pear:					
Stored for 106 days at 31° F.	13.00	107.4	2.1	0.31	14.8
Stored for 106 days at 31° F.; then ripened for 7 days at 65°	21.47	1,195.9	23.4	5.76	24.6
Golden Delicious apple:					
Stored for 41 days at 31° F.	6.66	126.4	2.4	.22	9.2
Stored for 41 days at 31° F.; then ripened for 7 days at 65°	8.69	140.8	2.7	.29	10.7
Winesap apple:					
Stored for 38 days at 31° F.	9.81	101.4	1.9	.19	10.0
McIntosh apple:					
Stored for 45 days at 31° F.	8.69	98.2	1.9	.21	11.0
Jonathan apples:					
Soft scalded tissue	6.67	1,328.8	26.0	3.43	13.2
Sound tissue	7.79	297.4	5.8	.92	15.9
Carrot [1]	41.80	531.5	10.4	.00	.0
Banana [1]	42.18	498.3	9.7	.00	.0
Sweetpotato [1]	48.50	307.2	6.0	.00	.0
Potato [1]	41.39	149.4	2.9	.00	.0

[1] Obtained from local fresh fruit market.

Differences in the composition of the internal atmosphere of normal and soft scalded tissue of Jonathan apples are also shown in table 6. The scalded areas contained slightly less carbon dioxide than normal tissue, whereas their total volatile content was approximately 5 times that of normal tissue. Evidently acetaldehyde accumulation cannot be the only factor in this large increase of total volatiles in the soft scalded areas, because, as shown in table 6, acetaldehyde comprises approximately a similar percentage (13 and 16) of the total volatiles in both types of tissue. It may be of more than passing interest to point out that both in the development of off-flavor in Delicious and in the formation of soft scald in Jonathan, total volatiles accumulated in large amounts. In the former case much of this increase was due to the accumulation of acetaldehyde (table 5), whereas in the latter, other types of volatiles were involved.

A higher carbon dioxide content and the absence of acetaldehyde are the main features that differentiate the internal atmosphere of the banana and certain vegetables from those of deciduous fruits (tables 5 and 6). One would scarcely anticipate that as there shown, the volatile constituents of the carrot were greater than those of the ripe banana, or even that those in the sweetpotato were present in larger amounts than those in the Golden Delicious or in the common Delicious apple.

SUMMARY

A method and an apparatus have been described for the simultaneous measurement of carbon dioxide and total volatiles in the internal atmosphere of fruits and vegetables. These analyses can be made simultaneously from the same sample of plant tissue.

The method involves (1) extraction of the internal atmospheres by refluxing and aspirating the plant tissue in boiling distilled water for 2 hours in an air stream of CO_2-free air at 20 liters per hour; (2) absorption of the components of the internal atmosphere in two gas scrubbers in series, the first bearing activated sulfuric acid for the removal of organic volatiles and the second bearing standard alkali for the removal of carbon dioxide; and (3) the oxidation of the organic absorbates with ceric sulfate.

Applications of the method include studies of (1) the adsorptive capacity of oiled fruit wraps for volatile emanations; (2) the influence of storage temperature, ripening processes, and soft scald on the composition of the internal atmosphere of fruits; and (3) comparative analyses of the internal atmosphere of certain vegetables and deciduous fruits.

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FURTHER STUDIES OF COOL STORAGE AND OTHER FACTORS AFFECTING THE FORCING PERFORMANCE OF EASTER LILY BULBS [1]

By PHILIP BRIERLEY, *pathologist*, and A. H. CURTIS, *student aide, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

In previous experiments cool storage of Easter lily bulbs (*Lilium longiflorum* Thunb.) was most effective in hastening flowering when applied shortly after digging. Evidence from a small-scale trial with American-grown lilies during the 1938–39 season suggested that new-crop bulbs of a genetically early Easter lily variety, if dug early and subjected to optimum cool storage, might yield flowers for Christmas. Preliminary trials also suggested that bulbs dug before they were sufficiently mature could not be forced. Further experiments were therefore designed for the 1939–40 season to test the response to combined early digging and cool storage and to determine whether immaturity would assume practical importance in such a procedure. Preliminary trials to determine the effect of region of production on performance and the degree of damage associated with mosaic were also included. These experiments of the 1939–40 season are reported herein. Summaries of these experiments [2] and a report of the earlier tests [3] have already appeared.

EFFECT OF DATE OF DIGGING AND OF STORAGE TREATMENT ON TIME OF FLOWERING AND OTHER QUALITIES IN FORCED CREOLE EASTER LILIES

In Louisiana it has been customary to dig bulbs of the Creole variety in early August. Bulbs dug at this time in 1938 and stored shortly after digging at 50° F. for 5 weeks flowered by February 24, 1939. The primary purpose of the experiment described herein was to determine how best to produce satisfactory flowers for the winter season from new-crop bulbs. One hundred and fifty Creole lily bulbs were harvested in Louisiana on each of six dates: June 16, June 26, July 7, July 19, August 1, and August 16, 1939. Bulbs from each harvest were shipped to the United States Horticultural Station, Beltsville, Md., immediately after digging, and on arrival they were divided into 6 lots of 25 each, of which 5 lots were placed in controlled storage rooms and the sixth was planted in the greenhouse. Storage facilities were provided at the Arlington Experiment Farm, Arlington, Va., or at the Beltsville station. Temperatures in the storage rooms were controlled within a range of approximately ± 1° F.

[1] Received for publication October 15, 1941.

[2] BRIERLEY, PHILIP. COOL STORING EASTER LILY BULBS TO HASTEN FLOWERING. Florists' Exch. 96 (19): 10–11; (20): 11–12, illus. 1941. See also Florists' Rev. 88 (2267): 21–24, illus. 1941. Also South. Florist and Nurseryman 51 (10): [3]–4; (11): [4]–6, illus. 1941.

[3] ——— EFFECT OF COOL STORAGE OF EASTER LILY BULBS ON SUBSEQUENT FORCING PERFORMANCE. Jour. Agr. Res. 62: 317–335, illus. 1941.

Treatments included (1) storage of bulbs at 50° F. for 5 weeks; (2) storage at 32° for 10 weeks; (3) planting without storage; (4) preliminary storage at 80° for 3 weeks in open trays followed by storage at 50° for 5 weeks; (5) preliminary storage at 80° for 3 weeks in open trays followed by storage at 32° for 10 weeks; and (6) planting after preliminary storage (at 80° for 3 weeks in open trays) only. Each lot was planted in the greenhouse as soon as the storage period was completed. Inasmuch as no two lots were planted on the same date, each was subjected to a different growing environment. Such confounding of season of growth with treatment is not considered objectionable because bulbs subjected commercially to any of these treatments would encounter similar seasonal conditions. The 50° storage treatment is the most effective stimulus to early flowering yet found. Bulbs were stored for 5 weeks at this temperature because earlier trials [4] had shown this period to be effective and a 10-week period injurious. During the longer period excessive rooting and sprouting may take place in storage. The standards of comparison provided were (1) 32° storage, representing commercial practice, and (2) planting in the greenhouse at once without storage. The 32° storage was continued for 10 weeks because earlier trials had shown that bulbs so stored bloomed earlier than those stored for 5 weeks at this temperature. In previous years planting without storage produced the best growth of any practice yet tested [4] in terms of height, number of flowers, and number of leaves. This procedure may therefore be regarded as nearly ideal for growth of the Easter lily but not commercially practical because of the long time (up to 9 months) required for flowering. All three of these practices were applied to bulbs (1) immediately after arrival and (2) after preliminary storage at 80° for 3 weeks, to determine whether bulbs dug while immature would continue to mature in warm storage and therefore respond better to storage at 32° or 50°.

All bulbs were grown in 6-inch clay pots. The soil and manure were composted for a year and then were lightened by the addition of one-fourth muck and fertilized with bonemeal. The soil for the entire experiment was prepared at one time and thoroughly mixed to insure as much uniformity as possible. Whenever possible the forcing temperature was held at 60° to 65° F. During early fall and late spring this temperature range was of course far exceeded during sunny days.

The experiment (experiment 1) included 900 bulbs, representing 6 dates of digging, 6 storage treatments, and 5 replications of 5 bulbs each. The 25 bulbs of each lot were distributed 5 to each of 5 greenhouse benches (blocks), and the 36 lots were randomized in each block. Because of space limitations it was necessary to grow these lilies with adjacent pots against each other, and competition between plants strongly affected the number of flowers and induced some blindness.

The mean values for each lot of 25 plants (5 from each of 5 blocks) and the variance analysis for 6 factors recorded are given in table 1. The effects of treatment and dates of digging on time of flowering are shown in figures 1 and 2.

[4] See footnote 3.

The slight differences in the size of bulbs supplied from the six dates of digging and also in the subdivision of these six lots into the six storage comparisons did not seem to have produced any important effects on the characters under study.

FIGURE 1.—Effect of storage treatment on time of bloom in Creole Easter lilies grown in 6-inch pots from bulbs harvested July 19, 1939: *A*, 80° F. for 3 weeks, then 50° for 5 weeks; *B*, 80° for 3 weeks, then 32° for 10 weeks; *C*, 80° for 3 weeks; *D*, 50° for 5 weeks; *E*, 32° for 10 weeks; *F*, planted on arrival. Photographed December 27, 1939.

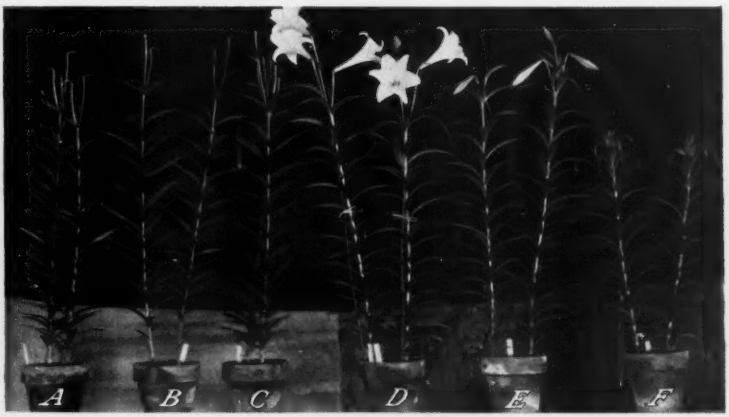

FIGURE 2.—Creole Easter lilies grown in 6-inch pots from bulbs harvested on six different dates and stored shortly after harvest for 5 weeks at 50° F.: *A*, Harvested June 16, 1939, flowered October 23; *B*, harvested June 26, flowered November 13; *C*, harvested July 7, flowered December 2; *D*, harvested July 19, flowered December 12; *E*, harvested August 1, 1939, flowered January 1, 1940; *F*, harvested August 16, 1939, flowered January 28, 1940. Photographed December 27, 1939.

TABLE 1.—*Effects of dates of digging and subsequent treatment on forcing behavior of Creole Easter lily bulbs produced in Louisiana*

EXPERIMENTAL DATA

Preliminary and storage treatment	Digging date	Planting date	Mean flowering date	Period from planting to—		Plants flowering [1]	Flowers per plant	Flower size (length × diameter)	Plant height	Leaves per plant
				Emergence	Flowering					
	1939	*1939*	*1940*	*Days*	*Days*	*Number*	*Number*	*Index number*	*Inches*	*Number*
Planted immediately	June 16	June 23	Apr. 5	83.4	287.2	22	5.7	25.0	43.6	145.3
	June 26	July 5	Apr. 3	79.2	273.4	21	4.9	25.1	42.0	143.2
	July 7	July 15	Apr. 10	72.8	269.7	20	4.5	25.7	43.0	143.2
	July 19	July 25	Apr. 12	61.5	261.9	23	5.0	24.2	39.8	144.3
	Aug. 1	Aug. 7	Apr. 10	51.2	246.6	23	6.2	24.9	43.3	143.7
	Aug. 16	Aug. 22	do	45.4	232.3	24	6.2	25.0	41.7	140.7
Mean				65.6	261.9		5.4	25.0	42.3	143.4
Stored immediately:			*1939*							
5 weeks at 50° F.	June 16	July 28	Oct. 23	18.1	87.0	21(+4)	3.7	24.5	27.4	54.7
	June 26	Aug. 9	Nov. 13	14.4	95.5	24(+1)	3.9	25.5	27.6	51.8
	July 7	Aug. 19	Dec. 2	17.2	105.5	25	6.4	26.4	29.7	54.2
	July 19	Aug. 29	Dec. 12	17.3	104.7	25	5.2	26.5	27.8	49.0
			1940							
	Aug. 1	Sept. 11	Jan. 1	13.2	112.1	25	5.1	25.8	31.2	55.1
	Aug. 16	Sept. 26	Jan. 28	17.4	123.9	25	4.1	24.6	31.8	58.8
Mean				16.3	104.8		4.7	25.6	29.2	54.0
10 weeks at 32° F.	June 16	Sept. 1	Jan. 31	35.0	151.9	15(+9)	4.8	25.5	38.1	73.3
	June 26	Sept. 13	Feb. 8	35.1	147.9	23(+2)	4.0	25.3	37.4	68.0
	July 7	Sept. 23	Feb. 18	35.3	148.5	23	3.8	25.6	38.1	67.8
	July 19	Oct. 3	Feb. 20	27.2	139.8	19	2.4	24.2	37.9	67.2
	Aug. 1	Oct. 16	Mar. 2	33.6	137.9	23	4.3	24.8	39.9	60.6
	Aug. 16	Oct. 31	Mar. 14	32.8	134.7	23	3.9	25.4	38.4	56.6
Mean				33.2	143.4		3.9	25.1	38.3	65.6
Preliminarily stored 3 weeks at 80° F.:										
Planted without subsequent storage.	June 16	July 14	Apr. 7	72.1	268.1	22	4.0	25.0	41.8	135.5
	June 26	July 26	Apr. 13	62.8	261.8	19	5.6	25.5	42.3	139.4
	July 7	Aug. 5	Apr. 8	57.8	247.1	24	5.0	26.3	43.3	144.2
	July 19	Aug. 15	Apr. 5	50.0	234.2	24	4.8	25.0	41.2	128.8
	Aug. 1	Aug. 28	Apr. 9	48.7	224.7	20	3.8	25.1	40.6	126.2
	Aug. 16	Sept. 12	Apr. 5	43.4	206.2	20	3.4	25.6	39.6	110.6
Mean				55.8	240.3		4.5	25.4	41.5	130.8
5 weeks at 50° F.			*1939*							
	June 16	Aug. 18	Dec. 23	32.4	127.3	20(+4)	5.1	27.7	34.9	70.6
			1940							
	June 26	Aug. 30	Mar. 9	46.1	191.6	21	3.7	25.0	38.4	86.0
	July 7	Sept. 9	Mar. 4	45.7	177.2	19(+1)	2.2	25.2	36.6	76.2
	July 19	Sept. 19	Feb. 7	27.4	141.4	25	4.2	26.1	33.4	65.7
	Aug. 1	Oct. 2	Feb. 26	29.0	146.7	24	3.1	25.3	34.2	65.0
	Aug. 16	Oct. 17	Mar. 19	32.9	153.9	22	3.4	24.8	38.8	70.2
Mean				35.6	156.3		3.6	25.7	36.1	72.3
10 weeks at 32° F.	June 16	Sept. 22	Mar. 5	52.1	165.2	22(+3)	3.5	25.6	40.4	69.8
	June 26	Oct. 4	Mar. 28	50.6	175.9	21	2.6	25.9	39.3	71.2
	July 7	Oct. 14	Apr. 3	58.2	171.7	15	1.0	26.5	39.6	66.8
	July 19	Oct. 24	Apr. 1	53.8	160.4	19	2.1	25.7	38.8	60.0
	Aug. 1	Nov. 6	Mar. 29	46.8	144.2	19	2.6	24.5	37.8	51.8
	Aug. 16	Nov. 21	Apr. 14	46.8	144.7	22	3.0	26.2	40.3	47.5
Mean				51.4	160.4		2.5	25.7	39.4	61.2
General mean				43.0	178.0		4.1	25.4	37.8	87.9

[1] 25 bulbs were planted in each lot; numbers in parentheses represent additional plants flowering far later than date given.

TABLE 1.—*Effects of dates of digging and subsequent treatment on forcing behavior of Creole Easter lily bulbs produced in Louisiana*—Continued

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square [1] for—					
		Days to emergence	Days to flowering	Flowers per plant	Flower size	Plant height	Leaves per plant
Blocks	4						
Diggings	5	1,035.05**	3,022.74**	1.57	2.61*	15.35*	795.39**
Storages	5	9,665.68**	109,523.60**	31.18**	2.83*	672.50**	45,170.40**
Diggings × storages	25	241.64**	1,289.50**	4.29*	2.62**	11.18**	190.02**
Blocks × diggings	20	15.32	40.27	3.35	.92	7.61	70.14
Blocks × storages	20	9.59	44.87	2.57	1.30	6.71	49.65
Blocks × diggings × storages	100	16.08	31.28	2.44	1.12	4.29	51.83
Pooled error	140	15.05	34.51	2.58	1.12	5.11	54.13
Total	179						

[1] *=Significant with respect to pooled variance indicated; **=highly significant with respect to pooled variance indicated.

Emergence was accelerated strongly by storage at 50° F. and less strongly by storage at 32°. Bulbs planted without storage treatment emerged more promptly as the season advanced, suggesting a progressive maturity. A slight hastening in emergence associated with preliminary storage at 80° as compared with the lots not preliminarily stored indicates that the bulbs continued to mature at this temperature.

Preliminary storage at 80° followed by storage at 50° or at 32° resulted in slower emergence than the respective cool storages alone.

Two points of interest appear with respect to the number of plants flowering. Flowering of all 25 plants was most often attained in lots stored at 50° F., which treatment induced prompt emergence after a short (5 weeks) period in storage. The lots stored at 32°, although emerging in approximately 1 month after planting, were handicapped by the 10-week storage period. The lots not stored were slow to emerge, although they were the first after each date of harvest to be planted in the greenhouse. The lots stored at 80° and those receiving combination treatments including 80° suffered to some extent both from the delay in storage and from late emergence. The reduction in number of plants flowering, or increase in the number of blind plants per lot, seems primarily a competition effect resulting from too close spacing of plants. In general, plants emerging early flowered normally, whereas those planted late or emerging late suffered, presumably for light, from the competition of more advanced adjacent plants. In previous forcing trials [5] Creole lilies spaced to receive ample light developed few blind shoots after prolonged 32° storage or when planted without cool storage.

A further peculiarity in number of plants flowering is indicated by the numbers in parentheses in table 1, column 7, which refer to individuals failing to respond to cool storage. Such plants did not flower with the majority in the same lots but bloomed 3 or 4 months later. These are scored as not flowering since they failed to respond to cool storage, although they flowered later at the usual time for the variety. It seems that bulbs that are dug too early are not sufficiently mature

[5] See footnote 3.

to respond to treatment. This may be the reason that 20 of the 150 bulbs dug on June 16, 3 of those dug on June 26, and 1 of those dug on July 7 failed to respond to cool storage. Bulbs planted on arrival and those stored at 80° F. apparently completed their maturity during the 2 to 3 months that they required for emergence. Thornton [6] and Thornton and Imle [7] reported similar immaturity effects in Easter lily bulbs grown at Yonkers, N. Y.

In accelerating flowering, storage at 50° F. was strikingly effective (table 1), as in previous trials. This treatment was more effective when applied immediately after digging than after a 3-week period at 80°. Bulbs dug early proved much more responsive to 50° storage than those harvested later. Bulbs stored for 10 weeks at 32° flowered much more promptly than bulbs not cool-stored, but they were slower to flower than bulbs from 50° storage. A period of 3 weeks at 80° followed by 10 weeks at 32° was less effective in hastening flowering than 32° alone. The lots not subjected to cool storage bloomed during the first 2 weeks of April regardless of the time of planting (June 23 to August 22). Bulbs receiving only 80° storage bloomed during the same period in April. This suggests a normal flowering season for the Creole variety, which is altered only by strong stimuli, such as cool storage.

With regard to actual dates of flowering it may be seen from table 1 that the 50° F. storage applied to bulbs from the six successive dates of digging yielded a reasonably even spacing of bloom from October 23 to January 28. Similarly, 32° storage produced flowers at intervals from January 31 to March 14. Thus, digging at different dates might serve as a means of controlling time of bloom. Such a means, however, is impracticable and other methods of timing are required.

Lilies from 50° F. storage flowered on significantly shorter stems than those from other treatments. Plants in lots not cool-stored were tallest, but not significantly taller than those in lots receiving 80° storage only. Lilies from 32° storage and those from the combination storage treatments of 80° and 50° and 80° and 32° were intermediate in height between the lots stored at 50° and those planted on arrival. All Creole plants in this experiment grew taller than in similar experiments of previous years, those in the 50° lots averaging about 10 inches taller. Also, those in the 50° lot dug on August 16 flowered more than 2 weeks earlier than those receiving a comparable treatment in the previous year. Placing the pots on raised benches rather than on ground beds as in earlier trials may have been responsible for these differences.

The data for number of flowers are misleading. The random distribution of the 36 lots through the 5 blocks (benches) and the close spacing led to severe competition between plants. The effects of this competition appear chiefly as reduced numbers of flowers and as blind plants, as previously mentioned in the discussion of number of plants flowering. Reduction in number of flowers was notable in the lots planted at once because these were slow to emerge, and also in the 32° F. and 80° and 32° combination lots because these were delayed in planting by long periods in storage. The net effect is to show these other treatments at a disadvantage, since the lots from 50° storage emerged promptly, bloomed early, and their vigorous

[6] THORNTON, NORWOOD C. DEVELOPMENT OF DORMANCY IN LILY BULBS. Boyce Thompson Inst. Contrib. 10: 318-388, illus. 1939.

[7] ——— and IMLE, E. P. WHY A DWARF EASTER LILY? Florists' Exch. 94 (15): 9, illus. 1940.

growth shaded those receiving the other treatments. The data, however, may be considered fairly representative of what may be expected of 50° storage. In a previous season [8] 7- to 9-inch Creole bulbs produced 8 or 9 blooms in lots planted at once, 3 or 4 flowers after 5 weeks at 50°, and 4 or 5 blooms after 10 weeks' storage at 32°. In the trial under discussion, 5 bulbs in one block of the lot dug August 16 and planted at once produced 12 flowers per plant, showing a capacity to bloom equal to that of Creole bulbs forced in previous years.

No satisfactory explanation can be offered at this time for some significant differences found in size of flowers.

Number of leaves is markedly reduced by preplanting cool storage. This evidently reflects the earlier differentiation of flowers in lilies from cool storage. Flower buds are laid down terminally on the axis and prevent differentiation of additional leaves. Bulbs from 50° F. storage produced only 49 to 59 leaves before flowering, but lots not stored developed 141 to 145 leaves. Plants from 32° storage also showed a sharp reduction in number of leaves, and as such plants were very little shorter than nonstored plants they had conspicuously longer internodes. Following combination-storage treatments the number of leaves per plant was lower, or the change from vegetative to reproductive development was earlier than in the lots not cool-stored.

Results of variance analysis (table 1) show that storage treatment produced a very marked effect on time of emergence, time of flowering, plant height, number of leaves, and number of flowers, and a slight but significant effect on size of flowers. The effect of storage on number of flowers was less marked than in previous trials, apparently because competition suppressed some flowers in nonstored lots. Dates of digging also show a marked effect on time of emergence, time of flowering, and number of leaves because the bulbs proved more responsive to storage treatment at the earlier storage periods. Despite rather large and highly significant interaction between digging and storage treatments, the variance due to storage was significantly greater than that due to this interaction for the characteristics of time to emergence, time to flowering, plant height, number of flowers, and number of leaves.

EFFECT OF DELAYING COOL-STORAGE TREATMENTS ON FORCING PERFORMANCE OF CREOLE EASTER LILY BULBS

The Creole lily bulbs in the second experiment were handled similarly to those in the first experiment except that storage treatments were applied after some weeks in common storage in Louisiana rather than soon after harvesting.

Fifty bulbs were dug on the 1st and 15th day of each month from June 1 through August 1, and half the bulbs from each of these five dates of digging were stored at 50° F. for 6 weeks and half at 32° for 15 weeks. Lots 1 and 2, dug June 1, were placed in cool storage approximately 1 month after harvest. Lots 3 to 10 were all placed in storage August 15, approximately 2 to 8 weeks after their several dates of harvest. All lots were planted in the greenhouse immediately after removal from storage. The bulbs were smaller than those in the

[8] See footnote 3.

TABLE 2.—*Effect of delaying cool storage on forcing performance of Creole Easter lily bulbs*

Lot No.	Storage		Digging date	Planting date	Mean flowering date	Period from planting to—		Plants flowering	Flowers per plant	Flower size (length × diameter)	Plant height
	Temperature	Period				Emergence	Flowering				
	°F.	Weeks	1939	1939	1939	Days	Days	Number	Number	Index number	Inches
	50	6	June 1	Aug. 16	Nov. 12	8.2±0.31	87.8±1.61	12	1.7±0.22	19.6±0.86	19.5±0.78
					1940						
2	32	15	do	Oct. 18	Mar. 16	35.6±2.57	150.3±3.85	15	2.4± .32	25.0± .42	30.8±1.31
3	50	6	June 15	Sept. 26	Feb. 9	17.1± .64	136.2±1.28	17	1.3± .11	23.5± .90	23.8±1.16
4	32	15	do	Nov. 20	Apr. 16	44.8±1.49	148.1±1.54	8	1.5± .19	26.3± .67	32.5±1.86
5	50	6	July 1	Sept. 26	Feb. 15	19.2± .65	141.3±2.20	14	2.1± .23	25.4± .83	23.4±1.08
6	32	15	do	Nov. 20	Apr. 7	31.2±1.40	130.2±2.01	17	2.1± .26	24.5± .58	26.4±1.05
7	50	6	July 15	Sept. 26	Feb. 20	19.2±1.87	147.6±2.71	13	2.5± .22	24.5± .88	24.9±1.85
8	32	15	do	Nov. 20	Apr. 7	39.3±2.03	138.6±2.35	18	2.3± .49	23.8±1.00	24.8±1.59
9	50	6	Aug. 1	Sept. 26	Feb. 11	17.4± .61	137.9±2.62	15	1.4± .13	22.3±1.13	24.0± .85
10	32	15	do	Nov. 20	Apr. 4	29.3±1.35	136.3±2.27	11	1.4± .28	22.6± .83	24.5±1.64

previous experiment and carried a high percentage of severe types of mosaic. They also were subjected to competition effects resulting from close spacing.

The performance of these bulbs, particularly with respect to number of flowers produced, was very unsatisfactory, but a few points of interest developed (table 2). Because the number of plants producing flowers varied from 8 to 17 out of a possible 25 in each lot, the only analysis attempted was the computation of means and standard errors for those flowering. These afford a rough measure of the differences required for significance between lots.

Lot 1, stored at 50° F. from July 5 to August 16, proved very responsive, as did similar lots in the trials just discussed, emerging in slightly more than a week and flowering in less than 3 months. The corresponding lot 2, stored at 32°, emerged in 36 days and bloomed after 150 days. The four lots, 3, 5, 7, and 9, harvested at different dates but all placed in storage at 50° at the same time (August 15), responded uniformly. These emerged in 17 to 19 days and flowered in 136 to 148 days, or from February 9 to February 20, showing differences of low significance or none. Evidently the season at which cool storage was applied determined the response. The date of digging appears to assume importance chiefly because it is necessary to dig early in order to store early. It is notable that storage at 50° at a comparatively late date (August 15 to September 26, lots 3, 5, 7, and 9) was much less effective in hastening flowering than similar storage earlier (July 5 to August 16, lot 1). In sharp contrast with the optimum response to 50° storage in early season, bulbs from 32° storage showed like performance regardless of the season of storage, all these lots emerging in 29 to 45 days and flowering in 136 to 150 days. The effect of early season storage (July 5 to October 18, lot 2) at 32° is thus not greatly different from that of later storage. There is, on the contrary, a general trend toward more prompt bloom in successively later plantings as noted in the previous experiment.

Plants from 50° F. storage treatments were comparatively short, ranging from approximately 19 to 25 inches. In the three earlier harvested groups, 2, 4, and 6, the plants from 32° storage were taller than those in the comparable 50° lots, but in the two later harvested groups, 8 and 10, they did not differ from the corresponding 50° lots, 7 and 9.

The flowers were few and small in this experiment, and data for both number and size showed variability not related to treatment. Disease content may have been responsible for part of this unsatisfactory performance. The effect of mosaic disease on forcing quality is discussed on page 231.

EFFECT OF PLACE OF PRODUCTION ON FORCING PERFORMANCE OF SEEDLING EASTER LILY BULBS

The bulbs in experiment 3 were nonselected mixtures of first-generation hybrids between commercial Easter lily varieties. They had grown for 2 or more years from seed, including 1 season in the production areas named. Bulbs were dug at Baton Rouge, La., Beltsville, Md., Raleigh, N. C., and Willard, N. C., on July 28, and at Los Angeles, Calif., on August 2, 1939. At Castle Hayne, N. C., they were dug in mid-June and held in common storage until late July. On August 11, 25 bulbs from each lot were stored at 32° F.

and 25 in common cellar storage at Beltsville. On October 20 all bulbs were removed from storage and planted in the greenhouse.

The planting design and the forcing practices were as described for the first experiment (p. 221). Again the number of flowers produced varied within lots, and there were many blind plants. In one block all five plants of the Castle Hayne sample from cellar storage were blind. Values for this group have been calculated according to Yates' [9] formula for missing plots. Much of the observed variability in number of flowers is attributed to crowding effects. The experimental data included in table 3 represent mean values from the five blocks in each instance. Variance analysis for the characteristics measured is also shown in table 3.

TABLE 3.—*Forcing performance of seedling Easter lily bulbs from six producing areas, stored at 32° F. or in a cellar from Aug. 11 to Oct. 20, when they were planted in the greenhouse*

EXPERIMENTAL DATA

Kind of storage and place of production	Mean bulb circumference	Mean flowering date	Period from planting to—		Flowers per plant	Flower size (length × diameter)	Plant height
			Emergence	Flowering			
	Inches	*1940*	*Days*	*Days*	*Number*	*Index number*	*Inches*
32° storage:							
Los Angeles, Calif.	8.8	Mar. 21	33.7	153.2	8.5	28.6	39.5
Baton Rouge, La.	8.9	Mar. 12	31.3	143.7	7.3	28.6	39.7
Beltsville, Md.	5.8	Apr. 30	44.4	192.9	5.7	24.9	28.4
Castle Hayne, N. C.	5.3	Apr. 5	33.1	168.5	1.5	31.6	34.1
Raleigh, N. C.	5.2	Mar. 24	29.9	155.7	2.2	31.1	33.2
Willard, N. C.	6.9	Apr. 4	26.6	166.7	3.4	29.5	31.2
Mean	6.8	Mar. 31	33.2	163.5	4.8	29.1	34.3
Cellar storage:							
Los Angeles, Calif.	9.7	May 11	20.5	204.2	8.1	26.9	49.7
Baton Rouge, La.	10.0	Apr. 21	23.6	184.3	10.3	25.6	44.5
Beltsville, Md.	5.3	May 14	42.2	207.4	3.3	24.9	31.9
Castle Hayne, N. C.	4.5	May 13	35.3	205.8	1.7	26.6	35.0
Raleigh, N. C.	4.1	May 4	38.1	197.0	2.2	27.8	34.8
Willard, N. C.	6.2	May 13	20.6	206.5	3.3	26.9	34.7
Mean	6.6	May 8	30.1	200.9	4.8	26.5	38.5
General mean	6.72	Apr. 19	31.62	182.16	4.79	27.75	36.40

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square [1] for—				
		Days to emergence	Days to flowering [2]	Flowers per plant [2]	Flower size [2]	Plant height [2]
Blocks	4					
Places	5	504.04**	1,484.24**	95.34**	27.12**	319.68**
Storages	1	142.30*	20,988.87**	.02	101.14**	252.56**
Places×storages	5	144.25**	370.19**	7.42*	7.00	27.89
Blocks×places	20	23.62	73.71	2.53	4.39	18.04
Blocks×storages	4	20.07	91.25	4.61	3.83	25.62
Blocks×storages×places	20	27.65	73.97	1.80	4.66	21.28
Pooled error	44	25.13	75.46	2.40	4.46	20.18
Total	59					

[1] * = Significant with respect to pooled variance indicated; ** = highly significant with respect to pooled variance indicated.

[2] 1 degree of freedom lost in a missing plot.

[9] YATES, F. THE ANALYSIS OF REPLICATED EXPERIMENTS WHEN THE FIELD RESULTS ARE INCOMPLETE. Empire Jour. Expt. Agr. 1: [129]–142. 1933.

Bulbs 7 to 9 inches in circumference were called for in the plan of the experiment, but only the Los Angeles and Baton Rouge samples permitted grading out 50 bulbs of approximately this size. Those from other sources were much smaller. Large bulbs are generally believed to flower somewhat earlier and to produce more flowers than smaller ones, but little is known of the relation of bulb size to other forcing characteristics.

Bulbs from the several production areas show differences in time of emergence. Those grown at Beltsville were slowest to emerge after both storage treatments. Lilies from Los Angeles and Baton Rouge came up more promptly after cellar storage than after 32° storage; those from Raleigh responded better after 32° storage; bulbs from Beltsville, Castle Hayne, and Willard showed no significant difference in effect of storage on emergence.

All bulbs stored at 32° F. flowered earlier than corresponding lots stored in the cellar. The advantage in favor of 32° storage is 37 to 41 days for bulbs from Baton Rouge, Castle Hayne, Raleigh, and Willard, 51 days for Los Angeles, and 14 days for Beltsville. Beltsville bulbs were clearly the least responsive to cool storing, and the Los Angeles bulbs possibly the most responsive. From cellar storage, 5 lots showed no significant departure from the mean requirement of 201 days to flower (May 8), the Baton Rouge sample alone showing distinct earliness (April 21). From 32° storage, 4 lots are grouped about the mean requirement of approximately 163 days (March 31), with Baton Rouge bulbs flowering earliest (March 12) and Beltsville bulbs latest (April 30).

With respect to height, lilies grown at Beltsville, Castle Hayne, Raleigh, and Willard are closely similar, but those from Baton Rouge and Los Angeles are taller. This difference is highly significant in plants from cellar storage and is lower but significant in plants from 32° storage. It is possible that this difference is related to the larger bulbs from Baton Rouge and Los Angeles. It is interesting to speculate whether there may be an additional tendency to tallness in lilies from the lower South as compared with a tendency to shortness in bulbs grown farther North.

No significant difference in number of flowers was found between storages, but between production areas there were several significant differences. In general, larger bulbs produced more blooms, as expected. Tall, early-flowering individuals also showed to advantage, since they suffered little from competition. Size of flower, which is not commonly shifted significantly by forcing methods, showed some tendency to higher values in plants that flowered early (March) and in plants that carried relatively few flowers.

Variance analysis (table 3) shows a highly significant effect associated with place of production on all characteristics measured. Between storage treatments are found differences in emergence, time of flowering, height, and size of flowers.

EFFECT OF MOSAIC DISEASE ON FORCING PERFORMANCE OF CREOLE EASTER LILY BULBS

The importance of mosaic in Easter lilies lies largely in its effect on forcing performance. No definite data are available on the degree of injury associated with the several mosaic types found in this species. Present field practice calls for roguing those types that seem destruc-

tive under field conditions. If data were available on the forcing performance of the several symptom types, roguing practices could be placed on a firmer basis.

The Creole lily bulbs included in this experiment represented three degrees of severity of the traditional lily mosaic or so-called necrotic fleck. The plants producing these bulbs were selected by D. L. Gill in the field in Louisiana during the growing season, when they were classified as (1) mild fleck, (2) medium fleck, and (3) severe fleck. The plants were labeled at this time, and after being dug they were stored for 10 weeks at 32°. Fifty bulbs of each class were then forced under conditions similar to those described for other experiments in this paper.

The data on performance are summarized in table 4. The standard errors afford a rough means of judging the significance of differences.

TABLE 4.—*Effect of three grades of mosaic on the forcing performance of Creole Easter lily bulbs dug on Aug. 1, 1939, in Louisiana, stored for 10 weeks at 32° F., and planted in the greenhouse Oct. 16, 1939*

[50 bulbs in each class]

Mosaic class	Mean flowering date	Period from planting to—		Flowers per plant	Flower size (length×diameter)	Plant height	Plants		
		Emergence	Flowering				Salable	Unsalable	Failing to bloom
	1940	*Days*	*Days*	*Number*	*Index number*	*Inches*	*Number*	*Number*	*Number*
Mild fleck	Mar. 19	50.9±1.06	155.4±1.72	4.6±.26	25.2±.24	33.0±.57	31	18	1
Medium fleck	Mar. 17	50.7±1.48	153.3±2.04	2.7±.22	24.0±.41	29.1±.74	12	32	6
Severe fleck	Mar. 26	56.7±1.49	161.7±1.99	1.9±.18	21.6±.59	22.1±.84	0	32	18

Since no disease-free lilies were included in this experiment, the mild-fleck class was chosen as the standard of comparison. Plants of severe-fleck history were slightly slower to emerge and to bloom, were much shorter, and bore fewer and smaller flowers. The differences in height and in number of flowers were particularly striking, both showing transitional values, in the medium-fleck class. Sixty-two percent of the mild-fleck and 24 percent of the medium-fleck class were judged salable according to standards of the Washington, D. C., market at Easter, 1940. This is in strong contrast with the severe-fleck class, in which no plants were classified as salable. The number of blind plants increased with the severity of the disease type, reaching 36 percent in the severe-fleck class. These results support the claim advanced earlier that severe mosaic affects height, number of flowers, and number of blind plants (see p. 227).

In view of the trend toward taller plants and to more and larger flowers in passing from severe fleck to mild fleck, one is tempted to infer that Easter lily seedlings are superior largely because they are virus-free. Commercial Easter lilies, including the Creole variety, are almost invariably affected with one or more viruses even though they show no symptoms.[10] The better commercial stocks carry low percentages of the necrotic fleck types included in this experiment (table 4) but are infected with mottling or latent viruses, which are

[10] BRIERLEY, PHILIP. PREVALENCE OF CUCUMBER AND TULIP VIRUSES IN LILIES. Phytopathology 30: 250–57, illus. 1940.

apparently less injurious than mild fleck. Data in table 1 from the commercial Creole lilies that were stored at 32° for 10 weeks and planted October 16 may reasonably be compared with the data in table 4. These Creoles grew taller than the mild-fleck plants, but did not differ from the latter in number or in size of flowers. The Baton Rouge seedlings from 32° storage (table 3) are also roughly comparable, in origin, bulb size, and treatment, with the mild-fleck Creoles of table 4 and with some of the Creoles of table 1. These seedlings approximated such Creoles in height and considerably exceeded them in size and number of flowers. The inference that virus-free seedlings force better than commercial stocks because they are disease-free is therefore tenable but not proved.

DISCUSSION

As shown in the first experiment, new-crop Creole Easter lilies may be flowered from November to January, depending upon the time of digging and upon the time of storing at 50° F. This winter market is now favorable because it is customarily supplied with bulbs of the Giganteum variety stored from the previous season. Although such bulbs deteriorate slowly in low-temperature storage (32° to 34°), their forcing capacity declines steadily with age. When forced after 9 to 12 months' storage, bulbs 8 to 10 inches in circumference seldom average as many as three flowers. The cool-stored domestic bulbs are thus competing with imported bulbs of lowest forcing performance.

Adaptability to forcing for the winter market is not peculiar to the Creole lily, although this is the only variety at present available in commercial quantities in the South. Seedling Easter lilies grown at Charleston, S. C., have been flowered in December. The suitability of a variety and of a production area for this early flower market is dependent largely on early maturity of the bulbs, for immature bulbs do not respond to 50° storage.

Little is known about the problem of maturity in Easter lily bulbs. It is clear that such bulbs when dug too early fail to respond to cool storage and develop so late after treatment that they represent a commercial loss. In the 1939 season most of the Creole bulbs tested were sufficiently mature by June 16, and all but one proved satisfactory for cool storing by July 7.

SUMMARY AND CONCLUSIONS

Creole Easter lily bulbs were dug in Louisiana on six dates: June 16, June 26, July 7, July 19, August 1, and August 16, 1939. As soon as the bulbs from each digging date reached Beltsville, Md., they were divided into six treatment groups: (1) Planted in the greenhouse at once; (2) stored at 50° F. for 5 weeks; (3) stored at 32° for 10 weeks; (4) stored at 80° for 3 weeks in open trays; (5) preliminarily stored at 80° for 3 weeks in open trays and then stored at 50° for 5 weeks; and (6) preliminarily stored at 80° for 3 weeks in open trays, and then stored at 32° for 10 weeks. The 36 treatment-digging combinations, each represented by 25 bulbs, were randomized through 5 greenhouse benches.

Bulbs stored at 50° F. for 5 weeks flowered in 87 to 124 days after planting; the mean dates of bloom were October 23, November 13, December 2, December 12, 1939, and January 1 and January 28, 1940.

Bulbs planted without storage flowered from April 3 to 12, 1940, after 232 to 287 days in the greenhouse. Lilies from 32° storage bloomed from January 31 to March 14, 1940, after 135 to 152 days in the greenhouse. Bulbs receiving only 80° storage flowered from April 5 to 13, 1940. Those from the combination storage treatment at 80° and then 50° bloomed from December 23, 1939, to March 19, 1940, and those stored at 80° and then 32° bloomed from March 5 to April 14, 1940.

Bulbs from 50° F. storage emerged and flowered earlier, flowered at lower stature, and produced fewer leaves than those from other treatments including the bulbs planted on arrival.

A few plants, chiefly from bulbs dug in June 1939, failed to respond to the stimulus of cool storage, but flowered in April 1940, at the same time as those receiving no storage. It is suggested that these individuals were not mature enough to respond to storage.

However, there is no assurance that these bulbs will mature on the same date in different years. If maturity is retarded by unfavorable conditions in some years, early digging and cool storing for winter bloom may lead to serious losses. Furthermore, no data are available on the effect of region of production on time of maturity, but it seems reasonable to assume that bulbs will be ready to harvest in the Gulf States before such bulbs can safely be dug farther North. It is common knowledge that the tops of Easter lilies die down earlier in the South, and this is the chief index of maturity in practical use at present. Immaturity effects similar to those reported here were encountered by Thornton and Imle [11] in Easter lilies of the Erabu and Giganteum varieties grown at Yonkers, N. Y. Similar performance, probably attributable to digging when immature, is sometimes seen in commercial Easter lilies of these varieties reaching this country from Japan in September and October.

Creole lily bulbs are more responsive to the stimulus of 50° F. storage in July than they are in August and September, and the intensity of this stimulus diminishes as the season advances (table 1). The season of maximum response to the 5-week interval at 50° follows immediately the season of immature response, when flowering is not at all hastened by such cool storage. Creole lily bulbs dug in June 1939 included individuals responding and not responding. The sharp transition from no stimulus offers the physiologists a problem in interpretation. Moreover, it offers a practical hazard to growers who seek maximum earliness of flowering.

In regard to the practical problem of spreading the season of flowering from November to January, the results of the second experiment carry a suggestion that deserves examination and further trial. It appears from these data that as a preliminary to 50° F. storage common storage in Louisiana is not different in effect from leaving the bulbs in the ground. If such is the case, a single digging in the first week of July in a normal year followed by 50° storage after successive intervals in common storage should yield successive periods of flowering similar to those attained by the more cumbersome method of successive dates of digging.

In a second experiment with Creole lily bulbs the time of harvest was varied, but the storage dates (except for lots 1 and 2) were the same. Bulbs from 50° F. storage flowered at nearly the same time

[11] See footnote 7.

regardless of the date of digging, and bulbs from 32° storage also were uniform in flowering season. The season when cool storage is applied is evidently of prime importance in determining the intensity of response; the time of digging is of importance only in that bulbs must be dug early to be treated. Storage at 50° was much less effective in hastening flowering when applied late in the season (August to September) than when applied in July.

Seedling Easter lily bulbs dug on approximately the same date in six different producing areas were forced after storage at 32° F. or in a cellar. From cellar storage Baton Rouge, La., bulbs were the earliest to bloom. From 32° storage Baton Rouge bulbs flowered earliest (March 12), and Beltsville, Md., bulbs latest (April 30), with those from Los Angeles, Calif., and from three points in North Carolina intermediate. Plants grown from bulbs produced at Beltsville, Md., Castle Hayne, N. C., Raleigh, N. C., and Willard, N. C., were similar in height, but plants from bulbs produced at Baton Rouge, La., and Los Angeles, Calif., were taller.

Creole lily bulbs from plants selected for three grades of severity of the necrotic fleck type of mosaic were compared. Bulbs from plants severely affected by fleck were slower to emerge and to bloom, much shorter, and bore fewer and smaller flowers than those from slightly affected plants. Thirty-six percent of the severe-fleck class failed to flower, and none of those that flowered was judged salable.

THE RELATIVE RESPIRATORY RATES AND HYGROSCOPIC EQUILIBRIA OF COMMON AND HULL-LESS OATS [1]

By P. E. RAMSTAD, *research assistant*, and W. F. GEDDES, *agricultural biochemist*, *Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station*

INTRODUCTION

The impression seems to be current among certain growers that hull-less oats (*Avena nuda* L.) are more susceptible to heating in storage than the ordinary varieties of oats (*A. sativa* L.). A series of respiration trials with Gopher oats and the hull-less variety South Dakota 165, grown at University Farm, Minn., in 1938, showed the respiratory rate of the former or ordinary variety to be appreciably higher than that of the hull-less oats at corresponding moisture levels. These results did not lend support to the idea that hull-less oats would heat more readily; on the other hand, they might be more hygroscopic than common oats and thereby exhibit a higher respiratory rate when stored at the same relative humidity. The experiments reported here were undertaken to recheck the respiratory rates of the two types of oats at similar moisture content and to determine their relative hygroscopicity.

MATERIALS AND METHODS

Unless otherwise noted, the varieties Gopher (common) and Nakota (hull-less) with test weights of 32 and 47 pounds per bushel, of high and approximately equal germinating power, grown at University Farm in 1939, were used. Respiratory rates were determined by the method used in previous studies at this laboratory. In brief, this method, which has recently been described in detail by Bailey,[2] consisted in incubating samples brought to moisture levels of approximately 11, 13, 15, and 17 percent at 37.8° C. (100° F.) for 4 days and determining the total carbon dioxide respired during this period by aspiration into standard barium hydroxide solution and back-titrating with standard hydrochloric acid solution. The moisture content of the samples was determined by a two-stage procedure, the first stage involving air-drying to hygroscopic equilibrium with the laboratory atmosphere, and the second, the determination of the residual moisture (after grinding in a Wiley laboratory mill to pass the 1-mm. sieve) by the vacuum-oven method of the Association of Official Agricultural Chemists.[3] In determining hygroscopicity, 3-gm. samples of the ground material were exposed in desiccators to atmospheres of known relative humidity, and the moisture content determined after constant weight had been attained. In adjusting atmos-

[1] Received for publication February 12, 1941. Paper No. 1886 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] BAILEY, C. H. RESPIRATION OF CEREAL GRAINS AND FLAXSEED. Plant Physiol. 15:257–274, illus. 1940.

[3] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 4, 710 pp., illus. Washington, D. C. 1935.

pheres to the desired relative humidities over sulfuric acid solutions, the data of Wilson [4] were employed.

The studies of Gilman and Barron,[5] Bakke and Noecker,[6] and others have emphasized the importance of molds and other micro-organisms in increasing the respiratory activity of oats and other grains at high moisture content. It seemed probable that micro-organisms might develop more readily on hull-less than on common oats. Accordingly, two subsamples of each variety were prepared for each moisture level. The respiratory rates of one set of subsamples were determined 4 days after wetting and the rates of the other set after storage for 30 days at 37.8° C. (100° F.). By storing the samples in this manner, an opportunity was provided for the growth of molds and other micro-organisms on the samples that were of sufficiently high moisture content.

EXPERIMENTAL DATA

These respiration studies were carried out in duplicate, and the mean values are recorded in table 1. For equivalent sample treatments, the hull-less variety, Nakota, exhibited an appreciably lower rate of respiration than Gopher. Molds were evident in the samples of both varieties brought to the two highest moisture levels (15 and 17 percent) and held for 30 days at 37.8° C. These samples exhibited a higher respiratory rate at the end of 1 month than originally, but the comparative rates for Nakota and Gopher were essentially unchanged. There is thus no evidence of a differential behavior of these varieties in regard to the development of micro-organic activity so far as is disclosed by the level of respiration.

TABLE 1.—*The respiratory rates of Gopher (common) and Nakota (hull-less) oats determined at 37.8° C. (100° F.), 4 and 30 days after being brought to different levels of moisture*

Moisture content (percent)	CO_2 per 100 gm. of dry matter per 24 hours—				Moisture content (percent)	CO_2 per 100 gm. of dry matter per 24 hours—			
	4 days after conditioning		30 days after conditioning			4 days after conditioning		30 days after conditioning	
	Gopher	Nakota	Gopher	Nakota		Gopher	Nakota	Gopher	Nakota
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
10.7		0.6		0.5	14.9	12.0		31.6	
10.8	0.9		0.5		15.2		4.5		22.0
12.8	2.2		.8		16.8	37.2		41.2	
13.0		.9		.6	17.5		33.4		39.1

The moisture content of ground samples of the two varieties in equilibrium with atmospheres of different relative humidity, given in table 2, reveals that Gopher is slightly less hygroscopic than the hull-less variety, Nakota. That this difference may be ascribed entirely to a lower hygroscopicity of the hulls as contrasted with the groats

[4] WILSON, ROBERT E. HUMIDITY CONTROL BY MEANS OF SULFURIC ACID SOLUTIONS, WITH CRITICAL COMPILATION OF VAPOR PRESSURE DATA. Indus. and Engin. Chem. 13: 326–331, illus. 1921.

[5] GILMAN, J. C., and BARRON, D. H. EFFECT OF MOLDS ON TEMPERATURE OF STORED GRAIN. Plant Physiol. 5: 565–573, illus. 1930.

[6] BAKKE, A. L., and NOECKER, N. L. THE RELATION OF MOISTURE TO RESPIRATION AND HEATING IN STORED OATS. Iowa Agr. Expt. Sta. Res. Bul. 165, pp. 317–336, illus. 1933.

was shown by studying the hygroscopic behavior of these two fractions prepared from Gopher oats by hand-hulling without previous parching or heat-treating. The hygroscopicity of the groats, which comprised approximately 75 percent of the kernel, was virtually the same as that of the hull-less variety, Nakota. At 70 percent relative humidity the groats contained 13.8 percent and the hulls 12 percent moisture.

The lower hygroscopicity of the hulls appears to provide a partial explanation of the higher respiratory rate of Gopher as compared with Nakota oats. At corresponding moisture levels, the groats of Gopher actually have a higher moisture content than those of Nakota and hence would be expected to respire at a more rapid rate. To investigate this point the respiratory rates of groats and the oats from which they were prepared were determined at a series of moisture levels as previously outlined. The groats were made from a commercial sample of untreated high-grade oats by means of a "green-oat huller" with the machine adjusted to produce the minimum mechanical and heat damage. The results, shown in table 3, reveal that at the same total percentage moisture content, whole oats have a higher respiratory rate than the groats, thus confirming the deductions drawn from the lower hygroscopicity of the hulls.

TABLE 2.—*The hygroscopic moisture of ground samples of Gopher (common) and Nakota (hull-less) oats in atmospheres of different relative humidity at approximately 25° C.*

Relative humidity (percent)	Equilibrium moisture content of—		Relative humidity (percent)	Equilibrium moisture content of—	
	Gopher	Nakota		Gopher	Nakota
	Percent	*Percent*		*Percent*	*Percent*
35	9.0	9.7	68	13.1	13.7
50	10.7	11.4	84	16.9	17.2
60	11.6	12.3			

TABLE 3.—*The respiratory rates of oats and the groats prepared therefrom, determined at 37.8° C. (100° F.) 4 days after being brought to different levels of moisture*

Moisture content (percent)	CO_2 per 100 gm. of dry matter per 24 hours for—		Moisture content (percent)	CO_2 per 100 gm. of dry matter per 24 hours for—	
	Oats	Groats		Oats	Groats
12.6		0.5	13.9	1.7	
12.8	0.9		14.5		6.2
13.7		1.4	15.8	27.9	24.7

DISCUSSION

These studies, while limited in scope, confirm the findings of preliminary studies undertaken previously with Gopher oats and the hull-less variety South Dakota 165, regarding the higher respiratory rates of Gopher as compared with hull-less oats at the same total moisture content. The higher respiratory rates of common oats, as represented by Gopher, even under conditions which favor mold

development, are the reverse of what would be expected on the basis of the impression held by growers that the hull-less varieties are more susceptible to heating in storage. Even when allowance is made for the higher hygroscopicity of Nakota, examination of the data presented here indicates that, when stored under the same conditions as regards atmospheric humidity, Gopher oats would still have a slightly higher respiratory rate.

It must be emphasized, however, that the respiratory rates have been compared on the basis of unit weight of dry matter per unit of time. Since the test weights of Nakota and Gopher oats were 47 and 32 pounds per bushel, respectively, there is approximately 50 percent greater weight per unit of volume in the case of hull-less oats. Thus, on a unit volume basis, the respiration of the hull-less oats would be greater than that of common oats if the air supply were unlimited. But there is also less air space per unit volume in the case of the hull-less oats, and under normal bin storage conditions the well known inhibitory effect of accumulated carbon dioxide on respiration would come into play sooner. These considerations make it appear quite possible that hull-less oats would decrease in viability more rapidly than common oats when stored in bins.

It would appear, then, that as far as danger from heating is concerned, hull-less oats could be safely stored at fully as high a moisture content as ordinary oats, but it would be necessary to investigate more thoroughly their susceptibility to micro-organisms before a definite conclusion can be reached. As far as storing oats for seed purposes is concerned, the present practice of preservation in bags stacked to permit free access of air should be continued until comparative data are available for the relative changes in germination capacity of oats stored in bags and bins over a period of time.

SUMMARY AND CONCLUSIONS

Respiration studies employing Gopher (common) and Nakota (hull-less) oats of high germinability and with test weights of 32 and 47 pounds per bushel, respectively, showed that, at the same total moisture content, the respiratory rate of Gopher per unit of dry matter was appreciably higher than that of Nakota, even under conditions which favored mold growth.

Gopher oats in equilibrium with atmospheres of different relative humidity were less hygroscopic (approximately 0.7 percent) than Nakota oats. This difference was shown to be due to the low hygroscopicity of oat hulls, which comprise about 25 percent of the kernel; at 70 percent humidity oat groats contained 13.8 percent moisture and oat hulls 12 percent moisture.

The higher respiratory rate of Gopher oats as compared with Nakota at the same total moisture content, is due in part to the low hygroscopicity of the hull and consequent higher moisture content of the respiring material, as shown by the fact that oat groats had a lower respiratory rate than the commercial oats from which they were prepared. At corresponding relative humidities, calculations indicate that Gopher oats have only a slightly higher respiratory rate than Nakota.

The data do not afford any basis for the assumption that hull-less oats would prove more susceptible to heating in storage, an assumption which has been more or less common among certain growers. Hull-less oats, however, have less interseed air space than common oats, and, under bulk storage, the carbon dioxide concentration in this space would tend to increase more rapidly. It is quite possible that these conditions would favor loss of viability in the bulk storage of hull-less oats at moisture levels which would provide a sufficiently high rate of respiration to result in the accumulation of carbon dioxide.

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DIAMETER OF FIBER IN DIFFERENT STRAINS OF ACALA COTTON [1]

By G. N. STROMAN

Associate agronomist, New Mexico Agricultural Experiment Station

INTRODUCTION

In the last few years the quality of cotton fiber has become increasingly important as a result mainly of a greater demand for better quality goods and the competition of both synthetic fibers and foreign growths. This has led to an increasing amount of research to determine the characters that go to make up quality in cotton fiber.

The main purpose of the investigation described in this paper was to determine what differences, if any, occur among related strains in respect to fiber diameter. Whether the method of swollen diameter is the best method for the breeder to use for fiber fineness, should he desire to know the fineness of his strains, requires further investigation. The diameter of fiber to be of great service would have to be used along with length, fiber weight per inch, strength, and other characters. There are several methods which could easily be adapted to any testing program for fineness of fiber (*2*, *4*, *8*, *15*).[2]

LITERATURE REVIEW

Pierce and Lord (*9*) first suggested the use of swollen diameter of fiber as an estimate of the intrinsic fineness of a cotton.

Hutchinson and Govande (*6*) showed that fibers of different species of cotton from different parts of the world varied greatly in hair weight and in swollen diameter. In certain samples they found significant correlation coefficients between hair weight per inch and swollen diameter, which seemed to show that swollen-fiber diameter could be used as a measure of fineness. In Malvi samples (*Gossypium arboreum* var. *neglectum* forma *bengalensis*) they found no significant correlation between hair weight and swollen diameter, but they did find a significant correlation between spinning value (or highest standard warp counts) and swollen diameter, the coefficient being -0.580, $P=0.01$. Between spinning value and hair weight they also found a significant correlation, namely, -0.597, $P=0.01$.

Hutchinson and Govande (*6*, *p. 43*) stated:

> Comparison of Pierce and Lord's (1934) [*9*] data for the relation between swollen hair diameter and hair-weight, with those available for Malvi cottons shows a much lower correlation in the latter than in the former. Environmental effects appear to have lowered the correlation between swollen hair diameter and hair-weight considerably, but not to have affected that between swollen hair diameter and spinning value to any great extent.
>
> * * * the environmental variances are in all cases very small compared with the crop variety variances. Environmental correlations also are usually small, so that in general the plant breeder is safe in assuming that much the greater part of the differences, he observes, is of genetic origin.

[1] Received for publication January 23, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 254.

For plant breeding purposes, the labour involved in the determination of hair-weight is prohibitive. The demonstration by Pierce and Lord (1934) [*9*] of the close relationship between hair-weight and swollen hair diameter opened up a new method of determining fineness, and the data given above for hair-weight, spinning value and swollen hair diameter in closely related Malvi strains tends to confirm the value of the method for estimating fineness in the kind of material with which the plant breeder works, though further work is necessary. * * * The development of a method whereby selection for high ginning percentage could be carried on without risk of developing a coarse lint is one of the most pressing problems in cotton breeding, and the determination of swollen hair diameter provides a possible solution.

Koshal and Ahmad (*7*), who have developed a method for determining swollen diameters, found in standard Indian cottons significant correlation coefficients between swollen diameter and fiber weight per inch, $r=0.746$; swollen diameter and standard hair weight, $r=0.839$; swollen diameter and spinning value or highest standard warp counts, $r=-0.800$. They state (*7, pp. 11–12*):

The correlation between highest standard warp counts and swollen diameter is negative and significant, consequently this fiber property can be utilised for the identification of cottons having different spinning values, or may be coupled along with the other fiber properties for predicting the spinning value of a cotton. For this purpose, however, it would be necessary to carry out swollen diameter measurements on a large number of cottons for which the other necessary data is available.

The present report is composed of three parts: (1) Swollen-diameter measurements of each length as pulled from the fiber sorter on 6 strains, 2 of which were grown in 2 different localities in 1938; (2) swollen-diameter measurements on the 1⅛-inch fibers of 12 strains of the advanced-strain test at State College, N. Mex., grown in 1938, and of 16 strains of a similar test grown in 1940; and (3) measurements of ribbon width, thickness, and number of convolutions of the 1⅛-inch length fibers of 4 strains and the relationship of these 3 characters in each strain.

MATERIAL AND METHODS

Samples of cotton (*Gossypium hirsutum* L.) taken from each plot of the advanced strain test were used. To obtain the swollen-diameter measurements in experiment 1, a sample of 10 seeds was selected, 1 from the middle of 1 lock of each of ten bolls. This is the basic method of taking a sample to be placed in a fiber sorter for obtaining the distribution of different lengths of fibers by weight in a sample of cotton.

The lint on this sample of 10 seeds was combed out very carefully and placed in a Pressley hand sorter. Then a sample of each length was drawn from the sorter and placed crosswise on a microscopic slide, a cover glass was placed over it, and the fibers irrigated with an 18-percent sodium hydroxide solution according to the method of Koshal and Ahmad (*7*). After 10 minutes the slide was placed under the microscope with a Euscope attachment and 100 fibers were measured by the aid of a micrometer eyepiece. Koshal and Ahmad (*7*) found that to obtain an accuracy of about 4 percent of the mean diameter with odds of 99:1, tests of diameter must be made on 90 fibers. If two similar cottons are to be tested for accuracy, 200 fibers must be used. In experiment 1, the measurements were made on fibers of strains 1064, 1980, 1517, 2780, 2768, and Sh 5 grown at State College, and on strains 1064 and 1517 grown at Roswell, N. Mex.

In experiment 2 samples were prepared as described above and 100 fibers were measured from each plot of the advanced-strain test at State College in the seasons 1938 and 1940. Measurements were taken, however, only on the fibers that were 1⅛ inches long. The 1⅛-inch length has been used in the writer's uniformity of length work (*13*, *14*). Hawkins (*5*) has shown that the long fibers are more immature than those of medium length. The variance of the average measurement of the fibers of each strain was calculated by methods of Fisher (*3*) and Snedecor (*12*). In experiments 1 and 2 the standard deviation of each set of measurements was calculated.

To determine ribbon width, ribbon thickness, and number of convolutions 50 fibers of 4 strains in the 1⅛-inch length group were used. Five measurements of ribbon width and thickness were made on each fiber and these were averaged to obtain the measurement for that fiber. The mean and standard deviations were calculated for each strain and simple correlations were run between each of the characters ribbon width, ribbon thickness, and number of convolutions. The convolutions were counted in a field of 1.6 mm.

TABLE 1.—*Mean swollen-diameter measurements and standard deviations for fibers of different lengths from 6 strains of Acala cotton grown at State College and at Roswell, N. Mex., 1938*

Fiber length (inches)	Mean diameter of fiber for strain No. —															
	1064, State College		1064, Roswell		1517, State College		1517, Roswell		1980, State College		Sh 5, State College		2780, State College		2768, State College	
	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation
	Microns	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*
1 3/8	23.6	2.8	23.4	2.5	22.6	1.9	22.7	2.8	20.8	2.5	22.6	4.3	21.6	2.1	22.8	1.9
1 1/4	23.9	2.1	25.1	1.8	23.2	2.2	23.4	3.0	20.9	1.9	22.7	2.4	22.4	2.5	23.0	2.1
1 1/8	23.0	2.2	23.1	2.4	22.6	2.3	22.8	1.9	21.7	2.4	21.9	2.8	22.3	2.2	24.1	2.2
1	23.5	2.0	23.4	2.3	22.8	2.6	23.1	2.4	21.5	2.4	23.0	2.3	22.8	2.1	23.6	2.1
7/8	23.6	2.0	22.6	2.7	23.1	2.6	23.2	2.5	22.3	2.3	22.4	2.2	22.5	2.0	23.6	2.5
3/4	23.9	2.4	24.4	2.4	23.5	3.0	23.6	2.9	22.4	2.5	23.2	2.9	23.1	1.8	24.3	2.5
5/8	24.0	2.5	27.3	3.1	24.9	2.8	23.6	2.5	23.3	2.7	23.8	2.5	21.7	3.1	23.5	2.1
1/2	24.4	2.3	26.2	2.5	26.7	3.6	24.2	3.2	23.2	2.8	25.1	2.7	23.5	2.0	23.6	2.0
3/8	26.4	4.8	25.7	3.0	25.1	3.4	25.8	3.8	23.4	2.6	27.0	3.3	24.2	2.8	24.0	2.9
1/4	24.8	3.7	26.5	2.4	24.7	2.6	24.1	2.7	22.0	2.3	24.7	4.2	22.4	2.2	23.4	2.8
1/8	25.6	4.1	24.5	2.6	24.1	1.8	23.8	2.7	23.2	2.4	25.5	3.7	23.4	2.8	23.1	2.6

EXPERIMENTAL DATA

SWOLLEN-FIBER DIAMETER

EXPERIMENT 1

The swollen diameters of each fiber length from ⅛-inch to 1⅜ inches of six strains are given in table 1. In figure 1, *A*, is shown a comparison of the mean swollen-diameter measurements of the different lengths of strain 1064 grown at State College and at Roswell, N. Mex. The diameters at State College are more regular and differ in other respects from those recorded for the cotton produced at Roswell.

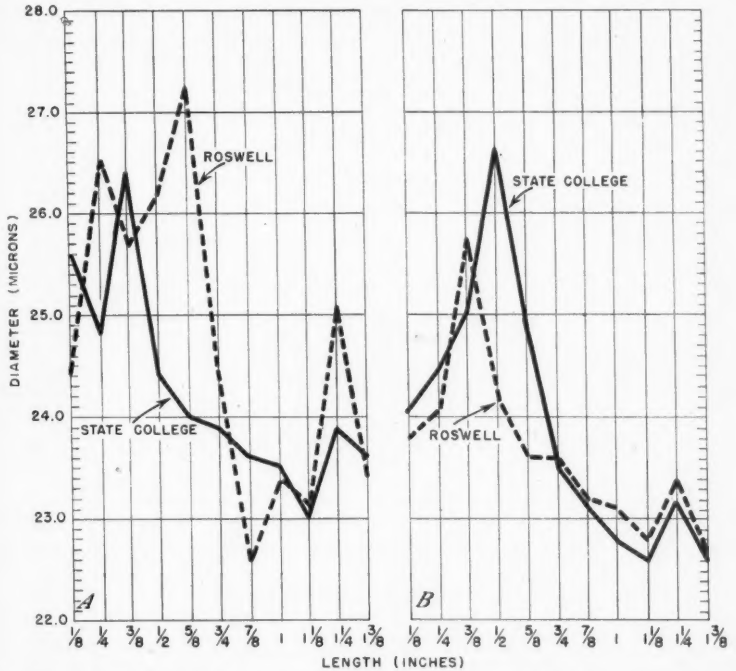

FIGURE 1.—Swollen-diameter measurements of different fiber lengths of strain No. 1064 (*A*) and of strain No. 1517 (*B*) grown at Roswell and at State College, N. Mex.

It will be noted in table 1 that the diameters of the longer lengths are, as a whole, smaller than the diameters of the shorter lengths. The 1⅛-inch length usually has the smallest diameter, and in measuring and comparing strains, the measurement of this length group would make for convenience. In New Mexico, where the cotton-breeding program has as one of its objectives the increase of the proportion of 1⅛-inch fibers in the total fibers of its cotton, interest in this length indicated that it should be used in making diameter measurements.

In strain 1064 the smallest diameter of any length is 23.0μ at the 1⅛-inch length, and the largest diameter is 26.4μ at the ⅜-inch length, a difference of 3.4 ± 0.53, which is highly significant. One

can readily see that with the standard deviation shown, a difference no larger than 1μ would be significant. Except in the case of strain 2768, the diameters tend to become larger with the shortening of the length. The data on the two strains 1064 and 1517, came

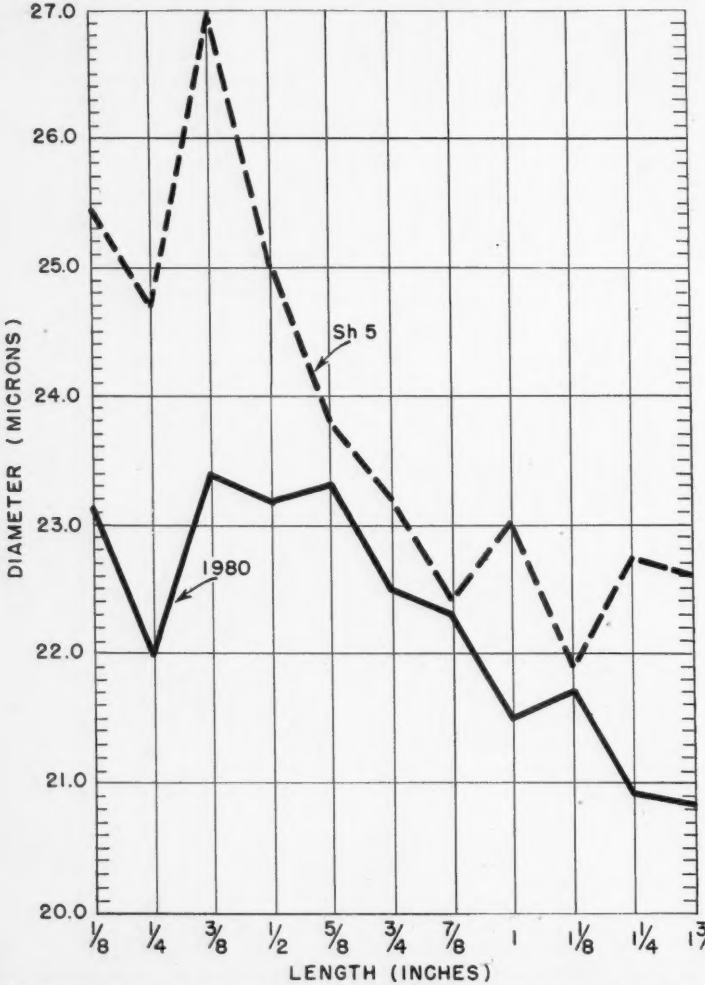

FIGURE 2.—Swollen-diameter measurements of different fiber lengths of unrelated strains Nos. Sh5 and 1980, both grown at State College, N. Mex.

from samples grown at State College and at Roswell. The tests on the other strains in 1938 came from samples grown at State College. Figure 1, *B*, shows a comparison of swollen-diameter measurements of one strain (1517) grown in two localities; the two curves are very similar. Figure 2 shows a comparison of swollen-diameter measurements of two unrelated strains grown in the same locality.

TABLE 2.—*Mean swollen-diameter measurements and standard deviations for 1⅛-inch fibers from each plot of the 12 strains of Acala cotton grown in the 1938 advanced test and of 16 strains of cotton grown in the 1940 advanced test at State College, N. Mex.*

12 STRAINS, 1938 TEST

Strain	Block 1			Block 2			Block 3			Block 4			Average diameter[1]
	Plot No.	Mean	Standard deviation	Plot No.	Mean	Standard deviation	Plot No.	Mean	Standard deviation	Plot No.	Mean	Standard deviation	
		Microns	*Microns*		*Microns*	*Microns*		*Microns*	*Microns*		*Microns*	*Microns*	*Microns*
1064	11	23.2	2.5	21	22.6	2.2	34	23.0	2.2	48	22.3	3.2	22.8
1980	3	21.8	2.3	24	21.7	2.4	26	21.5	2.4	38	22.0	2.2	21.7
1517	1	22.6	2.3	17	23.9	2.0	36	22.6	1.9	42	22.9	2.3	23.0
Sh 5	10	23.4	1.9	18	22.4	2.7	33	21.7	2.4	37	21.9	2.8	22.4
1450	9	20.6	1.8	22	20.9	2.0	25	20.9	1.7	43	21.1	1.9	20.9
1485	8	22.4	2.0	14	21.3	2.4	31	21.9	2.1	44	22.0	2.0	21.9
1493	12	21.3	3.0	16	23.7	2.6	32	23.3	2.4	41	23.0	2.3	22.8
1000	5	21.9	2.8	20	21.8	2.3	30	22.6	2.0	46	22.3	1.9	22.1
1757	[2]7	22.4	2.1	13	22.5	2.2	35	22.7	2.2	45	22.1	1.9	22.4
1497	2	21.4	2.2	19	22.7	1.8	28	22.0	2.1	47	22.5	2.4	22.1
California	4	23.0	2.0	15	22.3	2.1	27	22.3	1.8	39	22.3	1.8	22.5
N28–5	6	24.3	1.3	23	23.3	2.4	29	23.1	2.4	40	22.5	2.6	23.3

16 STRAINS, 1940 TEST

Strain	Block 1		Block 2		Block 3		Block 4		Block 5		Block 6		Average diameter[3]
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
	Microns	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*
2815	21.04	3.19	22.65	2.22	23.95	2.39	21.28	2.11	20.89	2.44	22.78	2.60	22.10
F. S. 1	21.27	2.91	22.62	2.13	23.32	2.08	22.48	1.92	22.17	1.84	22.02	1.76	22.31
2321	22.92	2.73	22.24	2.64	23.43	2.10	21.03	2.22	22.48	2.47	24.00	2.23	22.68
1517	22.51	2.38	23.83	2.40	23.59	2.33	23.82	2.48	23.97	2.22	23.71	2.57	23.57
1493	23.23	2.30	24.60	2.85	24.15	2.43	22.54	2.33	22.57	2.22	23.59	2.44	23.45
F. S. 2	20.65	1.98	23.38	2.31	23.68	1.95	23.13	1.84	23.01	2.32	22.66	2.39	22.75
1450	21.06	2.20	23.70	2.29	22.74	2.21	22.44	2.65	21.88	1.87	20.91	1.65	22.12
F. S 3	22.51	2.20	23.91	2.33	22.44	2.35	23.92	2.32	23.46	2.44	22.86	2.28	23.18
3231	23.64	1.90	25.00	1.90	21.87	2.42	21.51	1.74	23.86	2.24	21.91	2.27	22.96
2020	22.02	2.40	23.85	1.94	22.20	2.12	23.59	2.30	23.28	1.88	22.62	1.60	22.93
Deltapine 12[4]	24.06	3.24	25.00	2.61	23.95	2.15	24.07	2.33	23.16	2.61	24.30	2.68	24.09
3593	22.96	2.38	23.19	2.19	22.99	2.13	23.22	2.05	21.78	1.94	21.73	2.06	22.64
F. S. 5	22.41	2.05	23.46	2.11	22.53	2.15	23.25	2.15	23.26	1.83	23.29	1.89	23.03
1064	23.32	1.89	22.50	2.35	22.72	1.99	22.87	2.16	21.97	1.89	23.35	2.20	22.79
2314	22.35	1.94	24.04	1.98	22.32	2.38	24.10	2.09	23.25	2.84	23.58	1.75	23.27
1980	22.92	2.11	23.22	1.87	22.66	1.61	23.46	2.05	21.13	1.81	23.07	2.19	22.74

[1] Difference in average diameter required for significance 0.8.
[2] Mean of other 3 blocks used.
[3] Difference in average diameter required for significance 0.92.
[4] Not an Acala strain; seed derived from E. C. Ewing, Scott, Miss.

EXPERIMENT 2

In table 2 the mean diameter with its respective standard deviation is given for each plot of each strain in the advanced strain test at State College in 1938. In the last column is shown the average diameter of each strain, and in footnote 1, a difference for significance of 0.8μ.[3]

TABLE 3.—*Variance of the mean swollen-diameter measurements of the fibers grown in 1938 and in 1940*

Year and source of variance	Degrees of freedom	Mean squares	*F* values	Year and source of variance	Degrees of freedom	Mean squares	*F* values
1938:				1940:			
Blocks	3	0.1015		Blocks	5	2.4419	
Strains	11	1.6953	5.1	Strains	15	1.6656	2.63
Error	33	.3329		Error	75	.6323	
Total	47			Total	95		

The variance of the mean swollen-fiber diameters of both the 1938 and 1940 strains are shown in table 3.

The data in table 2 indicate in many cases significant differences between strains. In the 1938 test, strain N28–5 had the largest diameter, 23.3μ, and strain 1450 had the smallest, 20.9μ. This is a difference of 2.4μ, or 11.5 percent of the smallest strain.

Figure 3 presents a histographic comparison of all the measured strains grown at State College in 1938. At the right the strain 1980 mean measurement plus or minus the significant difference is shown for convenience of rating the strains.

The foregoing results suggest that if there are differences of 10 percent or more between related strains, the cotton breeder could improve his strain by using swollen-diameter tests of individual plants before they get through the breeding block. In fact, that is the point in the breeding program at which any test should be made, including the spinning test. It is easy to discard individual plants but costly to throw out a strain after it has been increased for distribution.

The mean diameter with its standard deviation for each plot of the advanced strain test at State College in 1940 is given in table 2. The average diameter for each strain is shown in the last column and the difference required for significance, 0.92, at the bottom of the table. All fibers used in the test, except those from Deltapine 12, were from strains of Acala. Strain 2815 had the smallest average diameter, 22.10μ, while Deltapine 12 had the largest, 24.09μ. The difference between these two, 1.99μ, is significant. The Acala strain having the largest diameter is 1517 with an average diameter of 23.57 or a difference from 2815 of 1.47, which is significant. Both 1517 and 2815 are selections from 1064, but neither 1517 nor 2815 differs significantly in average diameter from 1064. Both 1517 and 1064 have been tested for strength and found to have a strong fiber.

Figure 4 presents a histographic comparison of all the strains grown in the State College advanced strain test in 1940. A greater amount of variation is shown within strains in these data than in those for 1938. The amount required for a significant difference

[3] Standard error of mean difference=0.408.

between strains was 0.8μ in 1938 and 0.92 in 1940. The fiber diameters of the 1940 strains were also larger on the whole than those of 1938.

RIBBON WIDTH, THICKNESS, AND NUMBER OF CONVOLUTIONS

Data concerning ribbon width, ribbon thickness, number of convolutions, and the correlation relationships of these three characters in four strains are shown in table 4. Pope (*10, 11*), who studied these

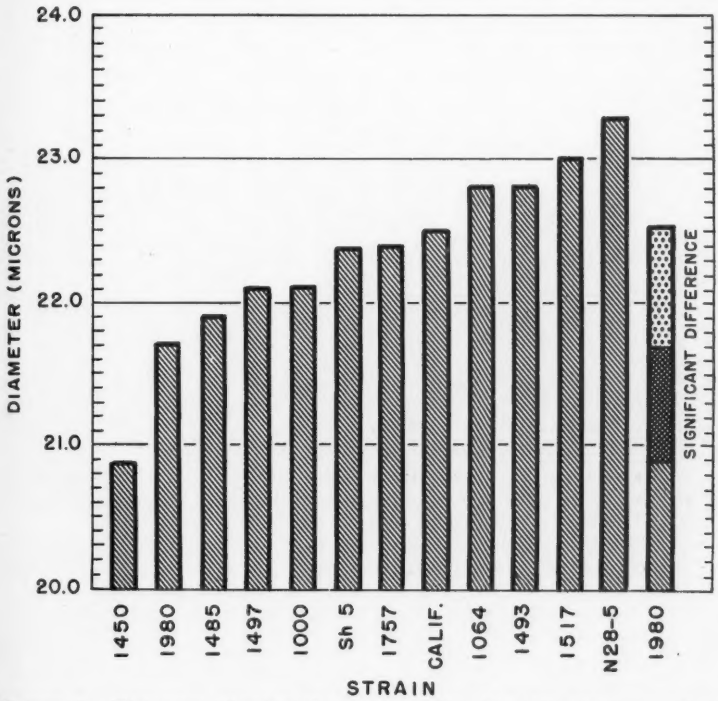

FIGURE 3.—Histogram showing the mean swollen-diameter measurements of fibers of the 12 strains grown in the advanced test in 1938, at State College, N. Mex.

characters, recommended a sample size of 100 to 200 fibers, but these data are taken on only 50 fibers.

The difference between the smallest ribbon width, 19.7μ, and the largest, 21.3μ, is 1.6 ± 0.49, which is significant. Strains 1064 and 1517, seed of which has been released to farmers in New Mexico, differ in width by 0.9 ± 0.47—hardly a significant value.

In thickness there is a significant difference between the smallest and the largest, namely, 0.9 ± 0.31.

In number of convolutions the difference between the highest and the lowest is 1.7 ± 0.73, which can be considered significant.

It is apparent from the foregoing analysis that the relationships as

shown by the simple correlation coefficients are not the same in all four strains. This behavior has also been observed by the writer in

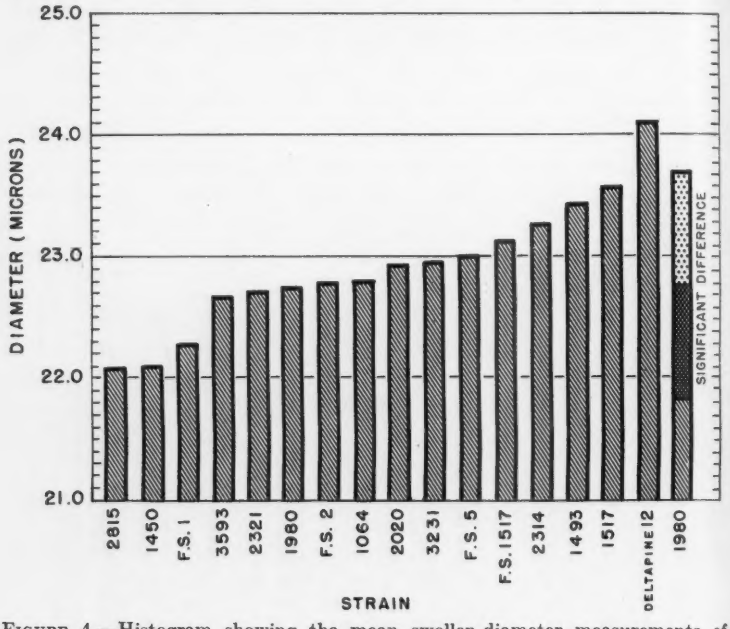

FIGURE 4.—Histogram showing the mean swollen-diameter measurements of fibers of the 16 strains grown in the 1940 test at State College, N. Mex.

a study of the relationship of characters where a large number of characters and strains were considered.

TABLE 4.—*Mean ribbon width, ribbon thickness, and number of convolutions per 1.6 mm.; the standard deviation of each and the simple correlation of ribbon width and thickness, ribbon width and number of convolutions, and between ribbon thickness and number of convolutions of 4 strains of Acala cotton grown in advanced tests at State College, N. Mex.,*[1] *1938 and 1940*

Strain No.	Ribbon width		Ribbon thickness		Number of convolutions		Correlation coefficients [2] of—		
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Ribbon width and thickness	Number of convolutions and ribbon width	Ribbon thickness and number of convolutions
	Microns	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*			
1000	19.7	2.3	11.9	1.3	15.9	3.5	0.49*	−0.12	−0.22
1517	20.4	2.1	11.1	1.0	16.7	3.8	.20	−.58*	−.01
1757	20.8	1.5	11.1	1.2	16.1	2.1	.20	−.35*	−.21
1064	21.3	2.6	11.0	1.8	17.6	3.8	.59*	−.44*	−.55*

N=50.
[2] Significant coefficient *.

DISCUSSION

The real object of these studies was to determine whether certain strains could be differentiated on the basis of diameter of fiber, and, if so, whether by the use of simple equipment it would be practicable to incorporate into the breeding program a system of testing progenies for fiber diameter. Swollen-fiber diameter and fiber weight per inch, as measures of fineness (*1*, *9*), have been shown to be of importance in spinning value (*6*, *7*, *16*), but the relation of shape of fiber and fiber weight per inch has not as yet been determined. When the cotton technologist has cotton of uniform fiber weight per inch, uniform diameter, uniform 40° angle, and uniform shape and length of fiber, he will be able to determine accurately the characters of raw fiber necessary to spin certain classes of textiles.

From the results here reported it can be seen that there are significant differences among strains of Acala cotton. It has been shown (experiment 1) that the different lengths of fibers from a given sample have different fiber diameters. For the comparison of strains as to fiber diameter it would not be practical to measure each length group so it seems logical to pick out for measurement one convenient length group. In New Mexico, as has been said, the breeding work on cotton has as one of its objectives the breeding for high uniformity of length of lint. The character used in the testing for this high uniformity of length has been the percentage of 1⅛-inch-plus fibers. In other words, in this program fibers 1⅛ inches long are important. The strains of Acala now distributed to farmers have about 25 percent of their fibers of this or longer lengths. Some new strains and progenies run as high as 60 percent of 1⅛-inch-plus fibers. Therefore at this station the 1⅛-inch group was selected as a suitable and convenient length to use in testing for swollen diameter of fibers. This measure would not, of course, represent the true diameter of the fiber, but it would be a figure that could be used in comparing one strain with another.

As regards ribbon width, ribbon thickness, and convolutions the Indian Central Cotton Committee (*1*) does not now test its standard cottons for these characters, because of the small contribution that these characters make to spinning utility. However, the diameter of fiber should be considered along with its strength, width of cell wall, and fiber weight per inch.

SUMMARY

Data were obtained on six different strains of Acala cotton to determine the swollen diameter of fiber in each group length of 1⅜, 1¼, 1⅛, 1, ⅞, ¾, ⅝, ½, ⅜, ¼, and ⅛ inches. The results showed that the shorter lengths had the largest diameters.

Measurements of swollen diameter on the 1⅛-inch length were made on 12 strains from the advanced test in 1938 and 16 strains in 1940 at the New Mexico station, and significant differences were obtained between strains. In 1938 the strain with the largest diameter was N28–5, with a mean fiber diameter of 23.3μ; strain 1450 had the smallest mean fiber diameter, 20.9μ. The difference between the two strains is 2.4μ, whereas 0.8μ would have been significant. Of all the Acala strains tested in 1940 No. 1517 had the largest mean fiber diameter, 23.57μ. Strain 2815 had the smallest mean fiber diameter,

22.10μ. The difference between these two mean diameters is 1.47, which is significant. This indicates that the cotton breeder could breed strains of cotton with small diameters.

A significant difference was found among strains in respect to ribbon width and thickness, and significant positive correlation coefficients between ribbon width and thickness were obtained in two of the four strains tested; significant negative coefficients were obtained between ribbon width and number of convolutions in three of the four strains, while between ribbon thickness and number of convolutions a significant negative correlation was found in only one of the four strains.

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